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GONADAL MATURATION IN THE COBIA, *RACHYCENTRON CANADUM*, FROM THE NORTHCENTRAL GULF OF MEXICO

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ABSTRACT Gonadal maturation of cobia, *Rachycentron canadum*, was evaluated by examining 508 specimens from its recreational fishery. Specimens were collected off southeast Louisiana to northwest Florida by hook-and-line during February through October 1987-1991. Fork lengths (FL) of these fish ranged from 580-1,530 mm, with corresponding weights of 2.0-43.5 kg. The female:male ratio was 1:0.37. Using a combination of oocyte size-frequency and histological assessment of many of the fish, we determined that females were ripe from May through September, with atretic oocytes occurring in some fish from July through October. Degenerating hydrated oocytes in July and October and the presence of resting ovaries in July suggest two major spawning periods; however, monthly gonosomatic indices peaking in May, followed by a steady decline, do not support that finding. Ovaries were placed into undeveloped, early developing, mid-developing, or late developing categories based upon oocyte size-frequency distributions. Developing ovaries had two or three modes of oocytes larger than 30 µm. Batch fecundity was estimated to be 2.6×10^6 to 1.91×10^8 oocytes, depending on the size of fish/ovaries. The smallest female with oocytes exhibiting vitellogenesis was 834 mm FL. This fish was 2 years old based its otolith evaluation. The smallest male with an abundance of spermatozoa in its testes was 640 mm FL and 1 year old based on otolith evaluation; smaller males were not examined. Females larger than 840 mm FL had vitellogenic oocytes in March and April. A few fish still had vitellogenic oocytes in early October, but none did by late October. When Gilson's fluid was used to assess ovarian tissue, the fresh weight of the tissue was reduced by 20% after being stored for 3 months. The diameter of oocytes shrunk about 25% in Gilson's fluid which was 11% less than those fixed in formalin, embedded in paraffin, and sectioned. Tissue sections from specific individuals, each demonstrating a variety of different developmental stages, were similar regardless of whether they were obtained from the anterior, middle, or posterior portion of either ovary.

Introduction

The cobia, *Rachycentron canadum* (Goode 1884), is a pelagic fish that is found throughout most of the warm ocean waters of the world, except for the Pacific coast of North America (Migdalski and Fichter 1983). In the western Atlantic Ocean, *R. canadum* occurs from Massachusetts to Argentina and is common in the Gulf of Mexico (Shaffer and Nakamura 1989). In the Gulf of Mexico (Gulf), cobia migrate from their wintering grounds off the southern Florida coast into the waters of the northern Gulf in late March and April and return to their wintering grounds in late autumn and early winter (Biesiot et al. 1994; Franks 1991b). However, a relatively large number of fish appears to remain in the northcentral Gulf during winter months at depths of 100-125 m (Howse et al. 1992). The cobia is a highly prized recreational species in the Gulf and U.S. South Atlantic Ocean. Most of the U.S. cobia landings come from Gulf waters (Shaffer and Nakamura 1989). Although most cobia are caught by recreational anglers, some are caught incidentally in U.S. commercial fisheries (Shaffer and Nakamura 1989).

Relatively little is known about the reproductive biology of cobia. Joseph et al. (1964) described eggs and juveniles collected from Chesapeake Bay and the nearby Atlantic Ocean and suggested that spawning occurred during summer. Richards (1967), also working in Chesapeake Bay, documented sexual dimorphism in size at maturity, presented evidence for spawning from late June through mid-August and postulated that multiple spawnings might occur. Dawson (1971) suggested that spawning occurred during spring in the coastal waters of the northern Gulf of Mexico. Finucane et al. (1978) reported evidence that cobia spawned off the coast of Texas in July and September, and Thompson et al. (1992) observed peak spawning in cobia from May through July in Louisiana coastal waters. Biesiot et al. (1994) described the biochemical changes in developing ovaries in cobia from the northern Gulf of Mexico and reported that spawning occurred during spring and summer.

Our study was undertaken to answer the following questions for cobia in the northcentral Gulf of Mexico: 1) what is the minimum size (length) of fish at maturity; 2) what is the temporal period of reproductive activity; 3) does the cobia spawn more than once per spawning season, and if so, what is the estimated batch fecundity of a female? We attempted to answer these questions through an analysis of oocyte size-frequency distributions, gonadal histology and the gonosomatic index (GSI).

MATERIALS AND METHODS

Cobia examined in this study were caught by hook-and-line in the recreational fishery off southeast Louisiana, Mississippi, Alabama, and northwest Florida during February through October 1987-1991. Additional gonad samples from six cobia caught from the Gulf side of the Florida Keys in January 1991 were used for histological evaluation only. In addition to those fish we caught, some were provided by recreational fishermen as well as state and federal fisheries agencies.

Fish were stored on ice from the time of capture until examined dockside or when received at coastal fishing tournaments. Fork length (FL) and total length (TL) were measured in mm, and total body weight (TW) was recorded to the nearest 0.1 kg. The pair of gonads was removed, placed in a resealable plastic bag, and stored in an ice slurry for up to 20 h. Total gonad weight was recorded to the nearest 0.1 g. A small subsample of each gonad was weighed to the nearest 0.1 g and fixed in 10% buffered formalin for histological examination. A second subsample of each ovary was weighed to the nearest 0.1 g and fixed in Gilson's fluid to facilitate estimation of oocyte numbers and size-frequency distributions.

A gonosomatic index (GSI) was calculated for both males and females: GSI = gonad weight/total fish weight) x 100.

Shrinkage of oocytes due to fixation was estimated by measuring the largest oocytes from fresh gravid ovaries, formalin-fixed-paraffin-embedded gravid ovaries, and Gilson's-fixed gravid ovaries. An estimate of the weight loss due to Gilson's fixative was determined by weighing a sample of fresh ovary at the time of collection, and then reweighing the same sample after 3 months in Gilson's fixative.

Ovarian tissue remained in Gilson's fluid for at least 3 months prior to estimating oocyte density and oocyte size-frequency distribution. A Bioquant® image analysis system was used to expedite oocyte counts and measurements.

The number of oocytes in an aliquot was determined using a counting chamber. Oocyte density was determined from the number of oocytes in corrected-weight aliquots of Gilson's fixed tissue and expressed as the number of oocytes per gram of fresh ovarian tissue. The total number of oocytes per female was obtained by multiplying the oocyte density by the total ovarian weight.

The frequency distribution of oocyte sizes was obtained by measuring the maximum distance across 100-200 randomly selected oocytes greater than 30 µm in diameter from an aliquot of Gilson's-fixed tissue. Presumptive batch fecundity, the presumed number of eggs released during each spawning episode, was determined on the basis of the percentage of oocytes appearing as the most advanced standing stock of oocytes in late developing ovaries.

Samples for histological analysis were embedded in paraffin from Hemo-De® xylene substitute, chilled, sectioned at 4 µm, stained with Gills hematoxylin, and counterstained with eosin-phloxine. Oocytes from the coverslipped slides were then staged according to sexual maturity. To determine whether the distribution of oocyte stages was homogeneous between ovaries and among anterior, middle, and posterior portions of each ovary; we examined histologically wedge-shaped samples from the wall to the lumen at those sites.

Size (length) at maturity was determined as the smallest fish which exhibited vitellogenesis or spermatogenesis. The ages of several fish examined during this study were determined as part of a concurrent study estimating age by otolith analysis (Franks et al. 1991a).

Statistical analyses were performed using Systat® software (Wilkinson 1990). Overall significance among group means was determined by the Kruskal-Wallis test ($P<0.05$); significance between pairs of means was determined by the Mann Whitney U-test using Bonferroni's correction ($P<0.05$). Batch fecundity data were transformed to logarithms ($\log_{10}$) to normalize the data before correlation analyses were performed.

RESULTS

A total of 508 cobia (374 females and 134 males) was sampled for reproductive analyses. The sex ratio of fish examined in this study, 1:0.36 (female:male), was representative of the sex ratio of cobia entered in fishing tournaments within our study area. Total weight and FL ranges among all specimens were 2.0-43.5 kg and 580-1,530 mm, respectively.

Seasonal pattern of maturation

All adult males ≥640 mm FL (N=134) and females ≥834 mm FL (N=361) were used for GSI calculations (Figure 1). Ovarian weights from the adult females ranged from 0.3% to 12.5 % of total body weight. Results of graphing GSI against month of collection revealed that the ovaries comprised an increasing proportion of body weight in spring, with a marked peak in GSI mean value of 5.0 in May, followed by a steady decline throughout summer and into autumn (Figure 1). Figure 1 also shows that the GSI for males is essentially the same as that for females.

Figure 2 shows the seasonal dynamics of vitellogenesis. Three of four females caught off Mississippi during March 1991 were in early vitellogenesis. In April, when cobia first appeared in near shore waters of the northern Gulf, all females ≥ than 834 mm FL were vitellogenic. The peak period of ovarian development occurred from April through June during which all females ≥834 mm FL were vitellogenic. Ovarian development in our samples decreased in late summer and autumn. Although a few fish were vitellogenic in early October, none were vitellogenic in late October.

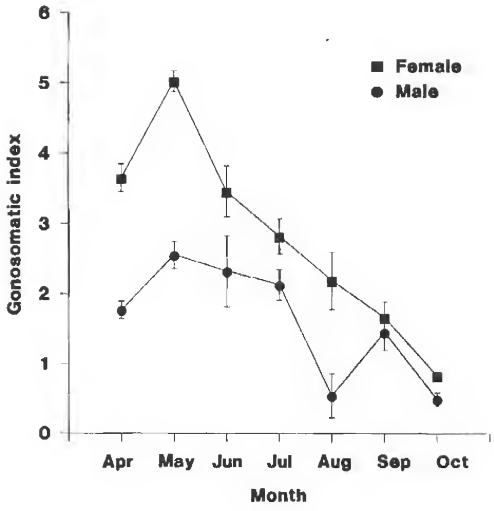


Figure 1. Gonosomatic indices for adult male (N=134) and female (N=361) cobia, *Rachycentron canadum* (means ± 1 standard error of the mean). Figure represents a composite of years 1987-1990.

Weight loss due to Gilson's fluid

The mean weight loss of ovarian tissue fixed in Gilson's fluid was 20% (S.E.=1.1%) of the fresh weight. This number was used as a correction factor for calculating the number of oocytes in fresh ovaries.

Shrinkage of oocytes due to fixation

The largest oocytes observed in fresh, well-developed ovaries were 950-1000 m in diameter. The largest oocytes in groups of large oocytes observed in Gilson's-fixed tissue from sites adjacent to those where fresh material was obtained from the same ovaries were 700-750 µm. Therefore, we estimate that diameter shrinkage due to Gilson's treatment was about 25%.

Examination of ovarian tissue from individual females, both by histological techniques and by Gilson's fixation, allowed for a more precise estimate of the relative shrinkage from the two treatments. Examination of 37 fish revealed that the diameter of oocytes fixed in Gilson's fluid was 11% (S.E.=3%) less than the diameter of oocytes treated by formalin fixation, followed by paraffin embedding.

Oocyte size-frequency distributions

Oocyte size-frequency distributions were estimated for 131 cobia. Inspection of the oocyte size-frequency distributions coupled with examination of histological sections of ovaries allowed fish to be placed into one of four groups representing various stages of ovarian development (Figure 3).

Group I, Undeveloped. Twenty-nine of the 131 fish for which oocyte frequencies were determined were placed into the first group. Figure 3 illustrates the oocyte-size distribution of a representative undeveloped fish. Undeveloped fish exhibited ovaries which contained the greatest proportion of small diameter oocytes. Fish placed into this group possessed ovaries with 90-100% of their Gilson-fixed oocytes with diameters less than 100 µm. The oocyte size-frequency distribution had a single mode of oocyte diameters between 50 and 100 µm, and all eggs were less than 250 µm. Nineteen of the 29 fish were examined histologically and confirmed to be inactive, i.e. not vitellogenic. The mean GSI of fish with undeveloped ovaries was 0.84 (S.E.=0.05). The mean number of oocytes per gram of ovarian tissue in these fish was 1.39×10^7 (S.E.=1.12×10^6) with the mean number of oocytes per female fish of 1.18×10^8 (S.E =1.78×10^7).

Group II, Early Developing. Forty-one of the 131 fish were placed in the early developing group. Figure 3 displays the distribution of oocyte sizes in the ovary of a fish in the early phases of vitellogenesis. Generally, fish in early development had ovaries with 50-90% of the oocytes smaller than 100 µm in diameter. The major mode was between 50 and 100 µm, but most of these fish had a small proportion of oocytes greater than 250 µm. Many fish showed signs of an additional minor mode of oocyte sizes, particularly in the 250-400 µm range. The mean GSI of fish in this group was 2.11 (S.E.=0.18). The mean number of oocytes per gram of ovarian tissue was 4.26×10^6 (S.E.=1.8×10^5), with a mean number of oocytes per female of 1.10×10^8 (S.E.=1.3×10^7).

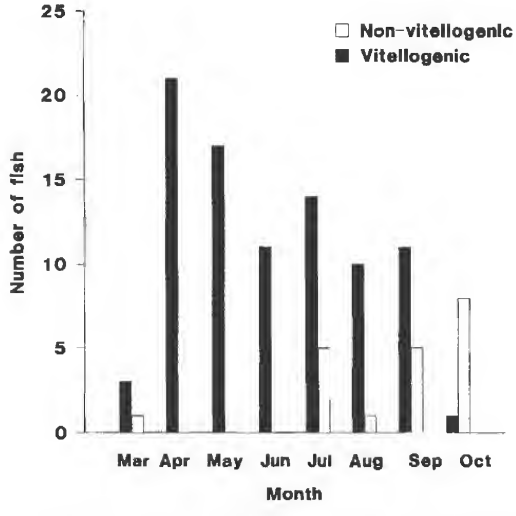


Figure 2. Monthly pattern of vitellogenic and non-vitellogenic female cobia, *Rachycentron canadum*, ≥ 834 mm FL. Figure represents a composite of years 1989-1990.

Not all fish could be correctly classified by their vitellogenic activity solely on the basis of oocyte size-frequency distributions. Seven fish which were initially classified as having undeveloped ovaries on the basis of oocyte frequencies were determined to be undergoing vitellogenesis based upon histological examination. No fish with their largest oocytes less than 150 µm were found to be developing. However, fish with their largest oocytes between 150 µm and 250 µm were either non-vitellogenic or undergoing vitellogenesis. All fish with at least one oocyte larger than 250 µm possessed oocytes with accumulated vitellin. Some of these fish could have represented post-spawning fish with residual vitellogenic oocytes.

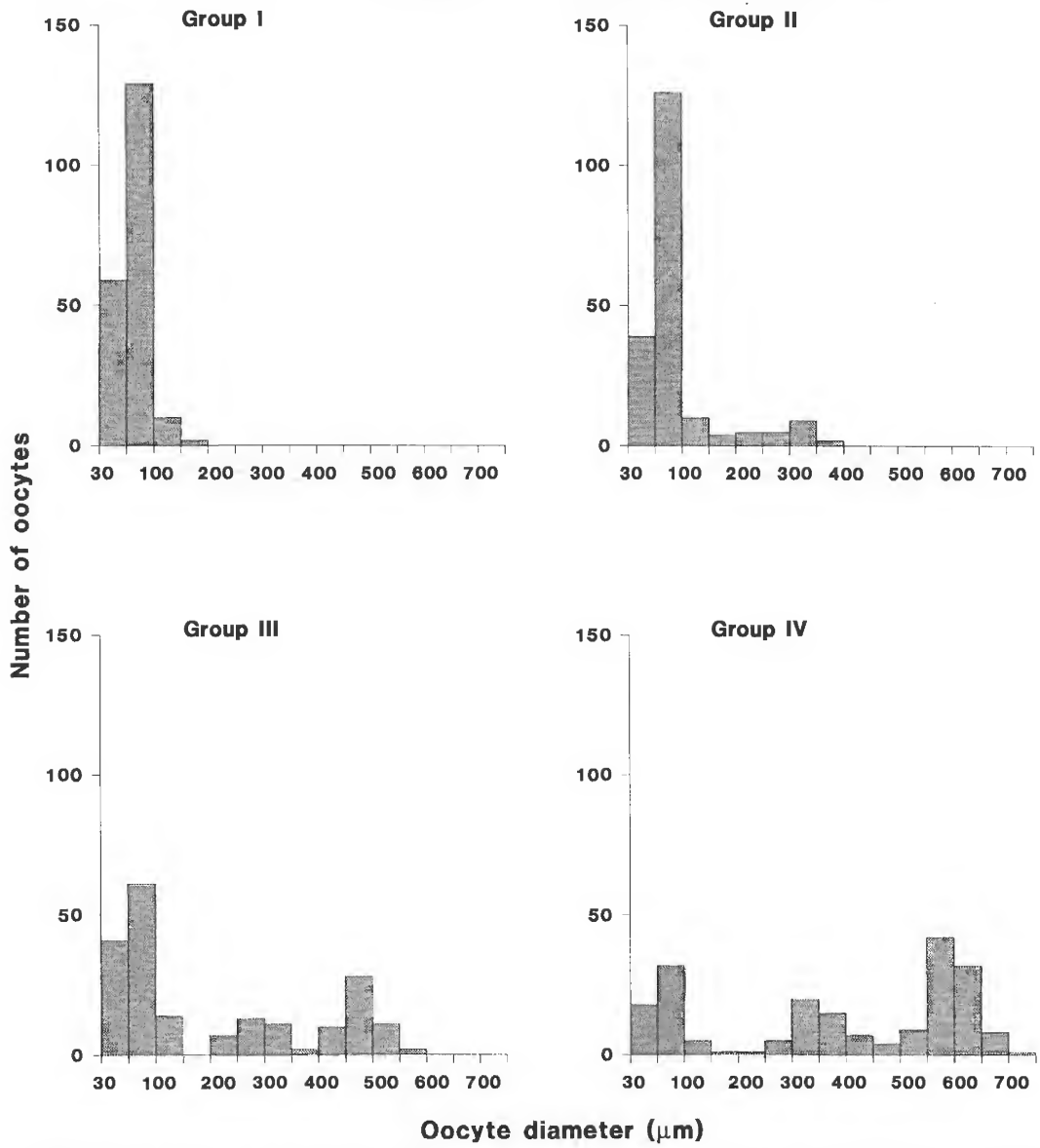


Figure 3. Oocyte size-frequency distributions of cobia, *Rachycentron canadum*. Group I: undeveloped ovary, Group II: early developing ovary, Group III: mid-developing ovary, Group IV: late developing ovary. Only oocytes 30 µm or greater were included in the groups.

Group III, Mid-Developing. Figure 3 depicts the oocyte size-frequency distribution of a fish belonging to the mid-developing group. Fish in this group exhibited oocyte-diameter distributions with a major mode at 50-100 µm. A distinct second mode was recognized at 400-450 µm or 450-500 µm. Some fish in this group displayed a third mode in the 250-400 µm range. The mean GSI of these females was 4.20 (S.E.=0.29). The mean oocyte density was 2.21×10^6 oocytes per gram (S.E.=5.40×10^5), with a mean number of oocytes per female of 1.47×10^8 (S.E.=2.47×10^7).

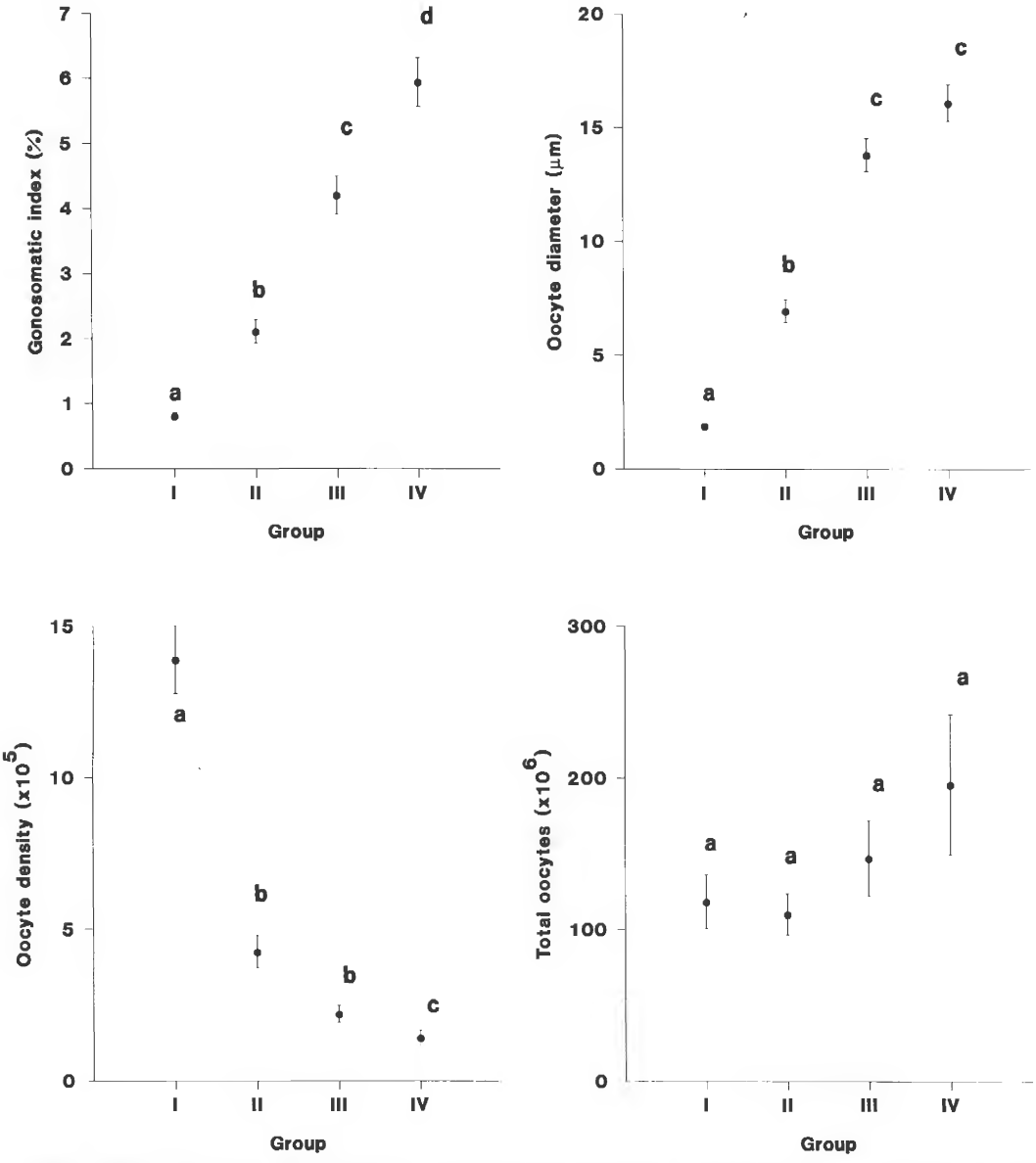


Figure 4. Comparisons of GSI, oocyte diameter, oocyte density and total number of oocytes among the four stages (groups) of ovarian development in cobia, *Rachycentron canadum* (means ± 1 standard error of the mean). Groups I-IV are as in Fig. 3. Means which share a letter are not significantly different from one another.

Group IV, Late Developing. The late developing group of fish possessed the most well-developed ovaries and were considered to be close to spawning (Figure 3). Twenty-three fish were placed in this group. The frequency distribution was distinctly bi-modal or tri-modal, with the most advanced mode in the 500 to 650 µm range. No running-ripe females or fish with hydrated oocytes were observed during our study. The mean GSI of these fish was 5.94 (S.E.=0.38), with a mean oocyte density of $1.40x10^6$ (S.E.=$2.34x10^5$) per gram of ovarian tissue. The mean number of oocytes per female was $1.96x10^8$ (S.E.=$4.64x10^7$).

A comparison of the four groups shows the relative size of the gonads, as a proportion of body weight (GSI) of female fish, increased as maturation proceeded (Figure 4). The differences among the four groups were statistically significant ($p<0.05$). Figure 4 further illustrates that the mean diameter of oocytes increased as maturation proceeded. The differences were significant between undeveloped (Group I) and early developing (Group II) ovaries, as well as between early (Group II) and mid-developing (Group III) ovaries, but the differences between mid-developing (Group III) and late developing (Group IV) ovaries were not statistically different. The density of oocytes per gram of ovarian tissue decreased as maturation proceeded and oocyte size increased (Figure 4). However, as Figure 4 indicates, there were no statistically significant differences among the groups in total number of oocytes per adult fish. Therefore, as maturation proceeded, there was little recruitment of new oocytes, and the size of the gonads increased to accommodate the increasing size of vitellogenic oocytes.

Histology

From some initial histological material not used for other analyses, we compared developmental stages in the left and right gonad of nine females and one male and found no significant differences in the stages of an individual. From four of those female fish, representing individuals with different stages of oocyte development, we examined histological sections from the anterior, middle and posterior of both ovaries and found no significant differences in the presence of stages among those sites. Each ovary contained previtellogenic and one or more vitellogenic stages, occasionally with a somewhat patchy distribution of oocytes in specific stages. In the case of the mature male, the tubules were especially filled with spermatozoa in the central portion. The walls of the efferent ducts contained more ripening germinal cysts with early developing stages in the periphery of the middle section of the testes than in those at either end. In summary, any section from a gonad provides a good indication of its developmental stage.

Figures 5-18 illustrate the developmental stages and features in cobia ovaries, and Figures 19-24 show those in cobia testes. Of the gonads of 94 females and 49 males examined histologically, those of 14 females and 9 males were from fish 860 mm FL or less. Four of the females and two of the males were fish caught in the Gulf off the Florida Keys 18-19 January 1991; two of those females and one male fit into the <860 mm FL category.

Some females were ripe May through September. Atretic oocytes occurred from July through October in nine fish from southeast Louisiana to northwest Florida, and degenerating hydrated oocytes occurred in three fish in July and October from the same location. However, a few fish in mid-June through July had ovaries in the resting state (containing both Group I and atretic oocytes), indicating they would produce eggs again because all females in August and September had high numbers of well-developed eggs. All four fish collected in January from the Florida Keys had atretic oocytes and degenerating hydrated oocytes.

Size at Maturity

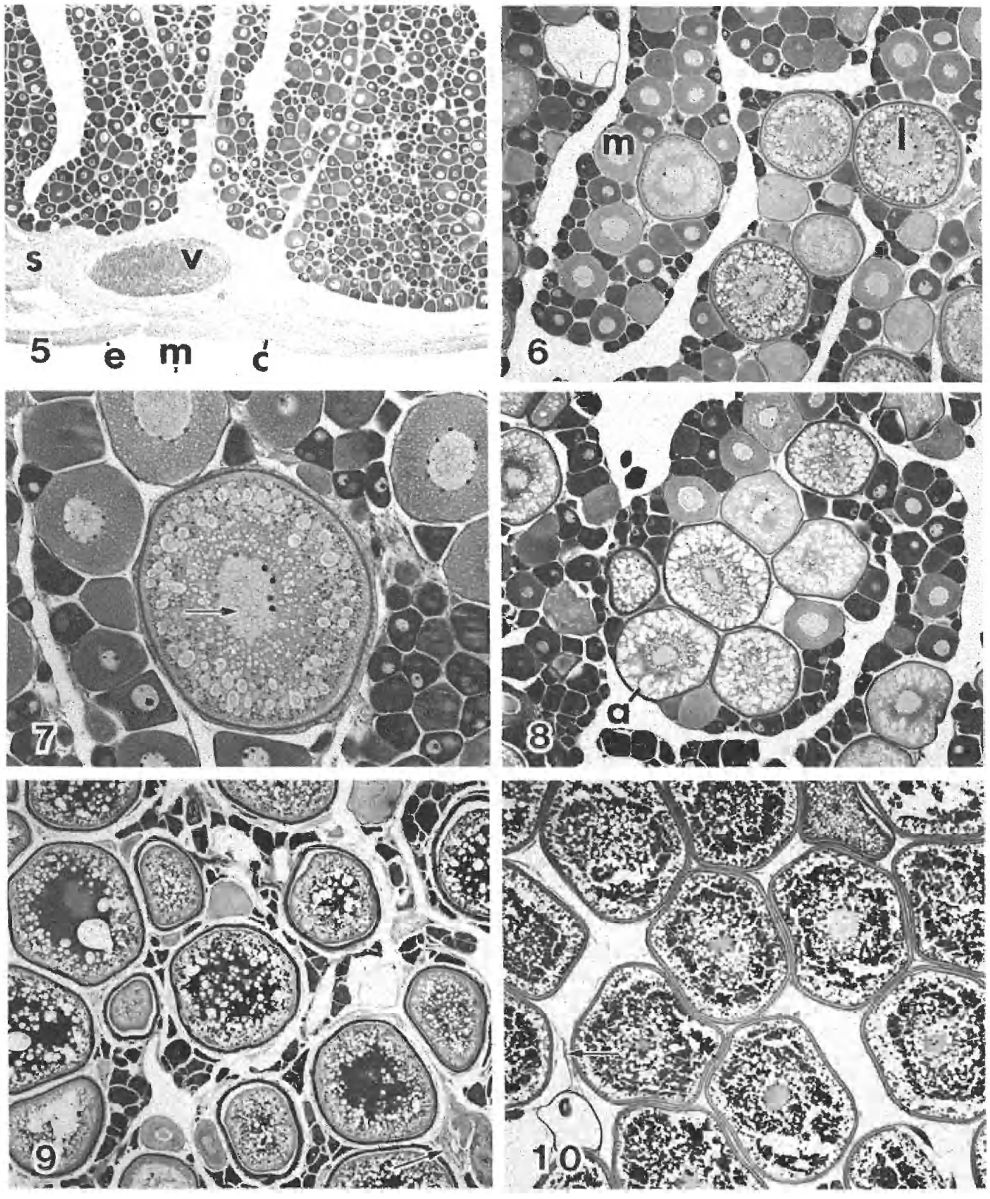
The smallest female exhibiting developing oocytes measured 834 mm FL and was determined to be 2 years old on the basis of otolith evaluation. Histologically, this was the smallest female fish with oocytes with a zona radiata and exhibiting vitellogenesis. Nine females (640 to 860 mm FL) examined histologically did not have developing oocytes. The smallest male exhibiting evidence of spermatogenesis was 640 mm FL. This particular male, 1 year old based on otolith evaluation, was not undergoing spermatogenesis although the tubules were filled with sperm. Actual onset of spermatogenesis may have occurred when this and other fish were smaller than 640 mm FL because no smaller male was examined histologically.

Spawning

The bi- and tri-modal oocyte size-frequency distributions observed for Groups III and IV (Figure 3) suggest that oocytes continued to be matured throughout the spawning season; however, the exact number of spawns and spawning frequency could not be estimated from these data.

The size of a batch spawn was estimated from the group of oocytes around the most advanced mode of oocytes in the 23 fish in the late developing group. The proportion of oocytes which were represented by the most advanced batch of oocytes ranged from 11 to 60% (mean = 28%; S.E.=3%). The estimated batch fecundity ranged from $2.6x10^6$ to $1.91x10^8$ oocytes (Mean=$4.8x10^7$; S.E.=$9.8x10^6$).

Among spawning fish, larger fish produced larger spawns. This is depicted by the significant positive linear relationship between the logarithm of batch fecundity and fork length and between the logarithm of batch fecundity and fish weight as measured for fish belonging to the late developing group (Figs. 25a, 25b). In addition, spawning fish with larger ovaries produce larger spawns. Figures 25c and 25d illustrate the significant linear relationship between the logarithm of batch fecundity and GSI, and between the logarithm of batch fecundity and total gonad weight of fish in late development.

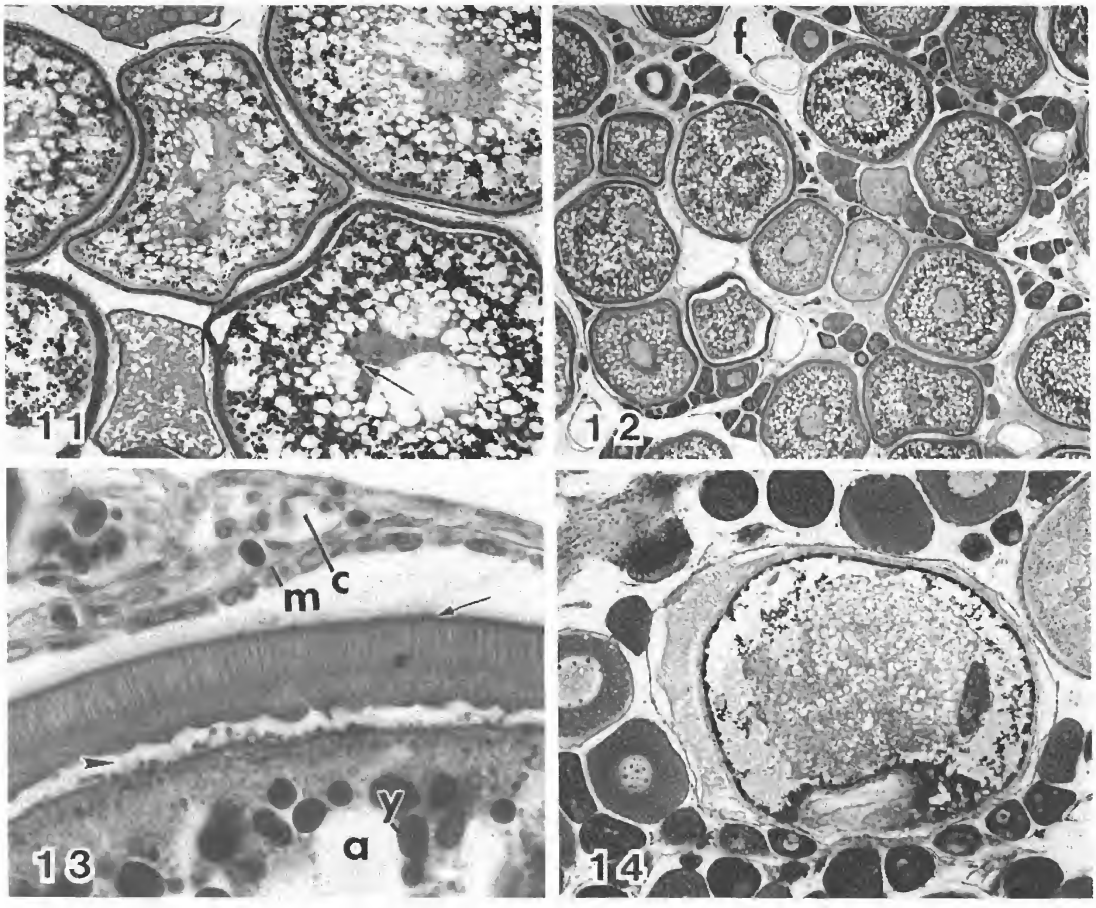


Figures 5-10. Sectioned ovarian tissue from cobia, *Rachycentron canadum*. Numbers included in all figure legends that precede fish data are slide numbers. 5. Ovigerous lamella of immature fish lined by an ovary wall (tunica albuginea). Note mesovarium (m) and thin outer squamous epithelium (e) bordering collagen layer (c) and thick layer of smooth muscle (s) containing large blood vessel (v) (2703, July, 710 mm FL, 3.4 kg, 18.6 gm ovaries, 1 year old). 6. Lamellae of early ripening ovary showing medium-sized (m) oocyte starting vitellogenesis (small cortical alveoli and peripheral nucleoli extruding from nucleus into cytoplasm) and relatively large (l) later staged oocytes among various sized oocytes and small oogonia (2671, April, 1000 mm FL, 12.4 kg, 92.0 gm ovaries, 2 years old). 7. Close-up of same ovary. Note lampbrush chromosomes (arrow) in nucleus of more developed oocytes. 8. Ovary in similar stage as that shown in Fig. 7 but with more extensive cortical alveoli (a) (2672, April, 1230 mm FL, 465.0 gm ovaries). 9. Ripe ovary with nuclei beginning to migrate marginally. Note postovulatory follicle (arrow) of released egg (2716, August, 1320 mm FL, 566.6 gm ovaries, 4 years old). 10. Ripe ovary similar to that in Fig. 9 but with few oocytes in early stages. Note ovigerous fold covered by squamous epithelium (arrow) (2839, August, 920 mm FL, 330.0 gm ovaries).

DISCUSSION

Our study suggests, as did those of Thompson et al. (1992) and Biesiot et al. (1994), that the reproductive season for cobia in the northcentral Gulf of Mexico is protracted and extends from April through early October with greatest activity occurring in the spring and early summer. This also parallels the reproductive activity of other Gulf of Mexico coastal pelagic fishes such as *Scomberomorus maculatus*, the spanish mackerel (Finucane and Collins 1986) and *Scomberomorus cavalla*, the king mackerel (Finucane et al. 1986).

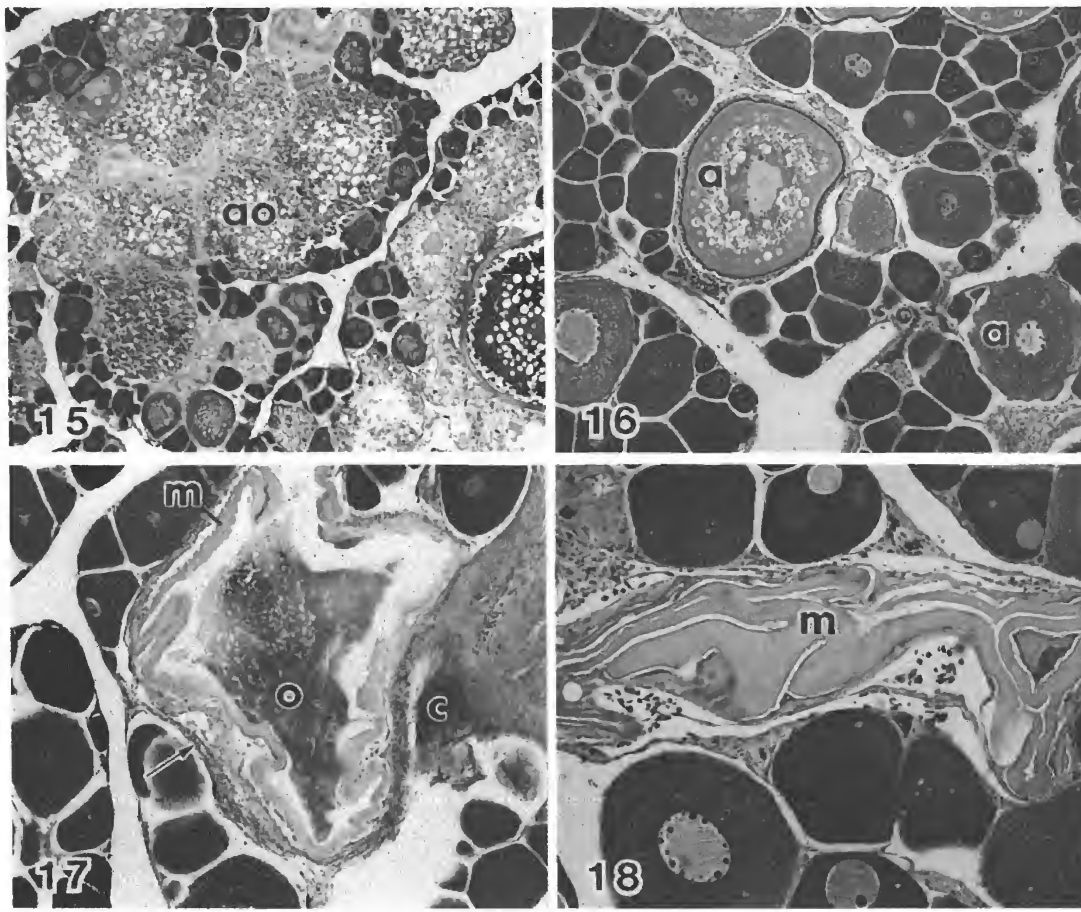
Richards (1967) reported the smallest mature female he examined from Chesapeake Bay was 696 mm FL, which is 138 mm shorter than the smallest mature female we observed. This discrepancy may reflect a slower growth rate for cobia in the cooler waters of the Chesapeake Bay area, rather than a regional difference in the age at maturation. Based upon scale



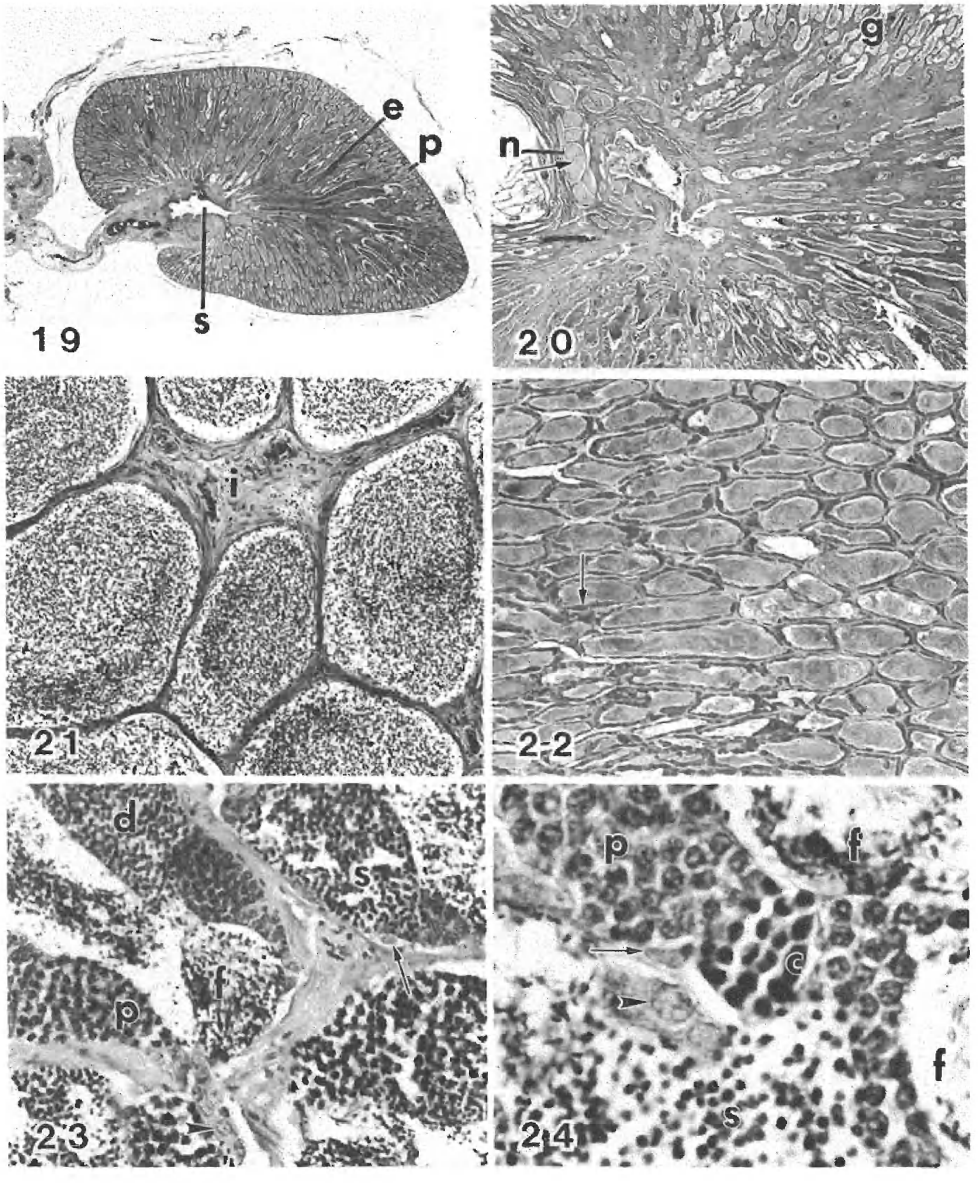
Figures 11-14. Sectioned ovarian tissue from cobia, *Rachycentron canadum*. Numbers preceding fish data are slide numbers. 11. Ripe ovary at beginning of spawning period showing ripe non-hydrated oocytes with irregularly shaped nuclei with extruding nucleoli (arrow) (2670, April, 1225 mm FL, 21.5 kg, 772.7 gm ovaries, 3 years old). 12. Ripe ovary in period between two apparent major periods of spawning. Note numerous empty follicles (f) (2700, July, 1231 mm FL, 21.8 kg, 584.6 gm ovaries, 3 years old). 13. Close-up of oocyte with the striated zona pellucida (zona radiata) separating (arrowhead) from oocyte cytoplasm. Note follicular wall consisting of outer granulosa containing lipid droplets and inner zona pellucida consisting of darker thin outer layer (arrow) and thick pale inner layer. The separated peripheral cytoplasm of the oocyte contains darkly staining yolk droplets (y) and clear cortical alveoli (a). External to the granulosa and divided by a conspicuous basement membrane (m) is the theca externa containing capillaries (c) (2700, July, 1231 mm FL, 21.8 kg, 584.6 gm ovaries, 3 years old). 14. Atretic oocyte in ovary of fish before initial spawning period. Note the degenerated marginal nucleus (2671, April, 1000 mm FL, 12.4 kg, 92.0 gm ovaries, 2 years old).

annuli, Richards (1967) surmised that females of 700 mm FL were 2 years old (in their third year of life). In the Gulf of Mexico, it is unlikely that a 700 mm FL female would be 2 years old, since 2-year-old females examined in this study averaged 850 mm FL (Franks et al. 1991a). Thus, 700 mm FL mature females collected in Chesapeake Bay by Richards (1967) may have been the same age as fish measuring about 850 mm FL in our study. The smallest mature male we found was 640 mm FL (age 1). Richards (1967) reported earliest maturity in males at 518 mm FL and age 1. Apparently males can mature when they are 1 year old, whereas females are not mature until 2 years of age.

The results of our study support Richards' (1967) suggestion that cobia spawn more than once during the spawning season. Richards (1967) reached his conclusion on the basis of finding fish with partially spent ovaries. We reached our conclusion because we observed group-synchronous oocyte maturation in fish collected during the spawning season, characterized by the presence of at least two distinct size groups of oocytes that had undergone vitellogenesis in the same ovary as well as postovulatory follicles (empty follicles) and atretic hydrated eggs in a few ovaries from July through October (and in January from the



Figures 15-18. Sectioned ovarian tissue from cobia, *Rachycentron canadum*. Numbers preceding fish data are slide numbers. 15. Ovary of different fish than in Fig. 12 but during same interspawning period, showing a resting ovary with an abundance of clusters of atretic oocytes (ao) (2695, July, 944 mm FL, 8.7 kg, 171.1 gm ovaries, 2 years old). 16. Early phases of atresia (a) of some oocytes in ovary of post-spawned fish after end of spawning (2723, September, 940 mm FL, 9.1 kg, 60.9 gm ovaries, 2 years old). 17. Degenerating hydrated egg in post-spawned resting ovary, showing fibrous capsule (c) of atretic follicle containing the hydrated oocyte (o) with its membrane (m) and containing an abundance of inflammatory macrophages and fibrocytes (arrow) (2731, October, 1110 mm FL, 141.2 gm ovaries, 3 years old). 18. Resting ovary of fish in winter with atretic follicle containing hydrated egg. Note homogeneous egg membrane (m) (2826, January, 991 mm FL, 10.9 kg, 120.0 gm ovaries, 2 years old).



Figures 19-24. Sectioned testicular tissue from cobia, *Rachycentron canadum*. Numbers preceding fish data are slide numbers. 19. Cross-section through area of ripe testis containing spermatic duct (s), radiating efferent ducts (e) filled with spermatozoa, and peripheral tubules (p) (2708, July, 640 mm FL, 2.4 kg, 5.8 gm testes, 1 year old). 20. Close-up of mature testis showing engorged efferent ducts and associated nerve bundles. Note enveloped capillaries (arrow) within some bundles (n) (2317, 960 mm FL, 8.2 kg, 92 gm testes). 21. Cross-section of mature tubules no longer lined with germinal cysts. Note interstitial tissue (i) among tubules containing different aspects of capillaries (2708, July, 640 mm FL, 2.4 kg, 5.8 gm testes, 1 year old). 22. Cross-and tangential-sections through tubules in developing testis. Note germinal cysts (arrow) lining sperm-filled tubules (2689, 1330 mm FL, 28.8 kg, 1192.3 gm testes, 7 years old). 23. Germinal cysts in various developmental stages in tubules surrounded by interstitial tissue associated with capillaries and Sertoli cells (arrow). Note spermatogonia (arrowhead), relatively large primary spermatocytes (p), relatively small secondary spermatocytes (s), smaller spermatids (d), and small spermatozoa (f) both in the cysts and in the lumen (2691, May, 970 mm FL, 12.4 kg, 290.5 gm testes, 4 years old). 24. Higher power showing spermatogonia (arrowhead), Sertoli cell (arrow), vacuolated primary spermatocytes (p), primary spermatocytes dividing into secondary spermatocytes (c), secondary spermatocytes (s), and spermatozoa with their streaming flagella (f) (2687, May, 1260 mm FL, 29.1 kg, 795.2 gm testes, 9 years old).

Florida Keys). However, the GSI values we obtained, along with those of Biesiot et al. (1994) and Thompson et al. (1992) during most years, did not support the late summer spawning activity.

Our estimates of batch fecundity are considerably larger than Richards' (1967) estimates of total fecundity. We estimated the size of a batch spawn to be between 2.6×10^6 and 1.91×10^8 eggs, with an average of 4.8×10^7 eggs per batch. Richards (1967) estimated total fecundity to be from 2×10^6 to 5×10^6 eggs per female, based on the total number of oocytes greater than 500 µm in diameter in the ovaries of six cobia. In our study, we used Gilson's fluid rather than formalin as in Richards' study (1967). Since we determined that oocytes shrink 11% more in Gilson's than

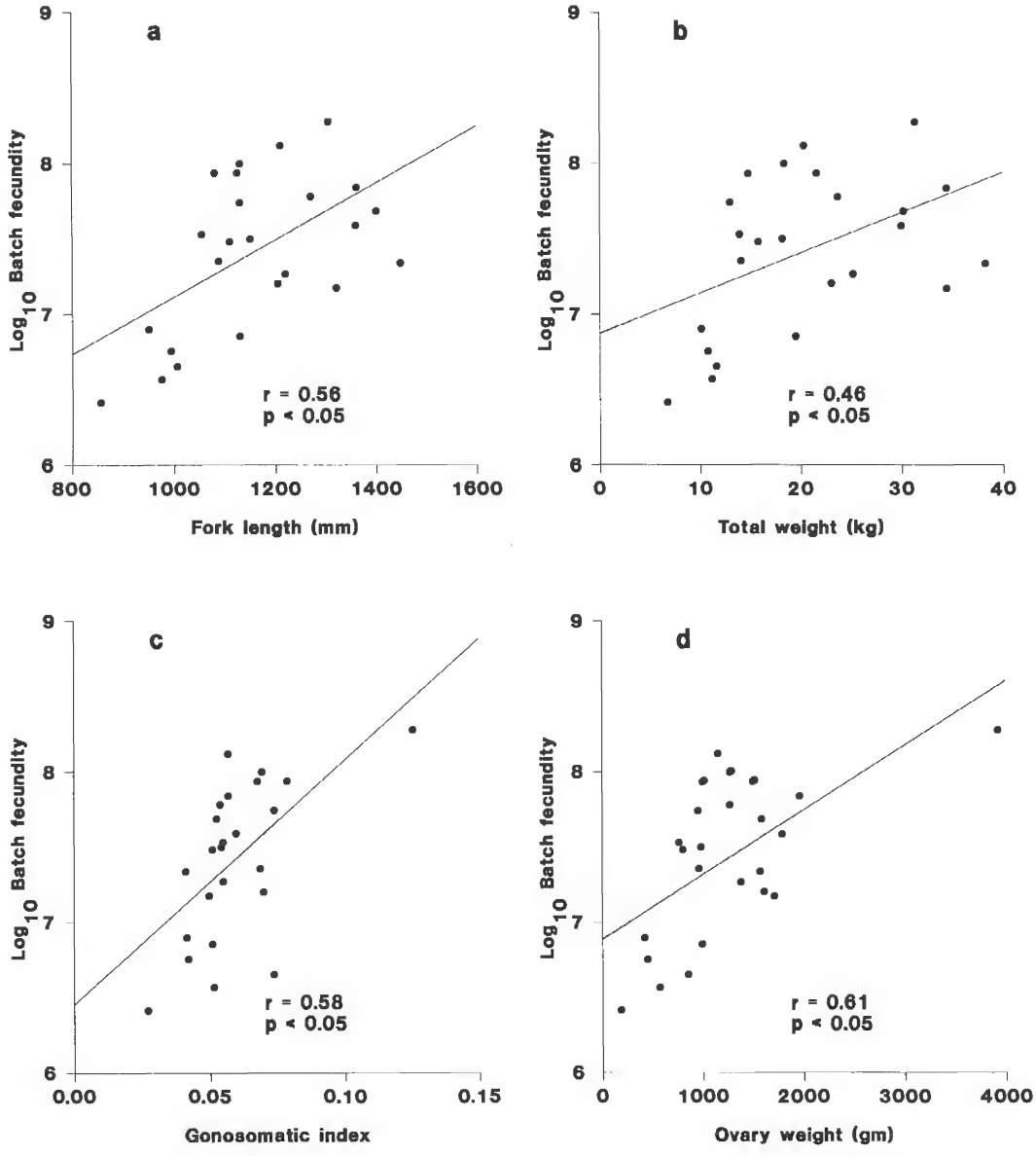


Figure 25. Relationship between batch fecundity and fork length (a), total weight (b), GSI (c) and ovary weight (d) for female cobia, *Rachycentron canadum*.

in formalin, we would probably have counted the same oocytes as Richards if we only consider eggs greater than 550 µm across. In late developing fish, oocytes greater than 550 µm generally constituted those used for our batch fecundity estimates. Those estimates are based on the advanced model group of developing oocytes and could overestimate fecundity if all eggs in the group are not released.

It is not straightforward to estimate the total seasonal fecundity of fish that spawn more than once per season without knowledge of the number of spawns per season. Although nonsynchronous formation of oocytes in the ovaries was observed, presenting strong evidence for multiple spawning (Hunter et al. 1992), we were unable to calculate spawning frequency (Hunter and Goldberg 1980; Hunter and Macewicz 1985; Hunter et al. 1985) because of the lack of recently hydrated oocytes and the relatively small sample sizes of fish from a single location over a one-year period. There appear to be some yearly variations in spawning, probably controlled in part by year-to-year temperature and locality fluctuations. Even though we never observed more than two advanced modes of developing oocytes over 30 µm in an ovary at one time by measurement, we are unable to conclude that cobia only spawn twice in a season.

This study showed that the total number of oocytes remained nearly constant as fish matured through the four gonadal developmental stages and spawned. In addition, the histological data and the oocyte size-frequency distributions indicated that some fish were close to spawning throughout the protracted spawning season. Therefore, it is assumed that recruitment of primary oocytes throughout the reproductive season and possibly continual transformation of primary oocytes into vitellogenic oocytes occurred throughout most of the reproductive season.

Whether cobia spawn during the day or at night is not well understood. Ditty and Shaw (1992) postulated that cobia spawn during the day because all larvae (N=74) examined from the Gulf, with one exception, were in similar late stages of development when collected during mid-morning. Behavior believed to be daytime spawning of cobia was observed off Panama City, Florida (Shaffer and Nakamura 1989). In the present study, we observed no fish with recently hydrated eggs, even though all examined fish were captured (by hook-and-line) during daylight hours. One explanation for the lack of fish with hydrated eggs in our study is that spawning cobia do not feed, as suggested by Richards (1967), and thus are not subject to capture by baited hook. Another explanation is that at least some cobia spawn at night. Some cobia may spawn far offshore as suggested by the abundance of eggs found in the Gulf Stream offshore from North Carolina (Hassler and Rainville 1975) and by reported observations of cobia spawning approximately 80 km off the South Carolina coast (Shaffer and Nakamura 1989). Cobia examined in our study were caught near mainland and barrier island beaches, in ship channels, over shallow water wrecks, and at petroleum structures located no further than 40 km offshore.

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FOOD OF COBIA, *RACHYCENTRON CANADUM*, FROM THE NORTHCENTRAL GULF OF MEXICO

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ABSTRACT The stomach contents of 403 cobia, *Rachycentron canadum*, caught in the northcentral Gulf of Mexico recreational fishery from April through October of 1987-1990 were examined. Cobia ranged from 373-1,530 mm in fork length. Of the 403 stomachs, 287 (71.2%) contained at least one identifiable prey taxon. Crustaceans, consisting primarily of portunid crabs, were the predominant food. Crustaceans occurred in 79.1% of the stomachs and comprised 77.6% of the total number of identifiable prey. The second most important prey category was fish which was dominated by hardhead catfish, *Arius felis*, and eels. Fish occurred in 58.5% of the stomachs but only accounted for 20.3% of the total number of prey. The importance of fish as prey increased with increasing size (length) of cobia, with the largest size class of cobia (1,150-1,530 mm FL) showing the highest percent frequency occurrence of fish prey (84.4%). There were no significant differences between the diets of male and female cobia. Species composition of the diet indicated that cobia examined in this study were generalist carnivores in their feeding habits and fed primarily on benthic/epibenthic crustaceans and fishes. However, the occurrence of pelagic prey provided evidence of diversity in the foraging behavior of cobia. Feeding in cobia indicated their dependence upon prey availability rather than upon a few specific food organisms.

Introduction

Rachycentron canadum, commonly known as cobia or ling, is a widely distributed, pelagic fish which occurs worldwide in tropical, subtropical, and warm temperate seas, except in the central and eastern Pacific Ocean (Shaffer and Nakamura 1989). In the western Atlantic, the cobia occurs from Massachusetts to Argentina (Briggs 1958), but is most common in the Gulf of Mexico (Migdalski and Fichter 1983), where it supports an important recreational fishery. In the Gulf of Mexico (Gulf) cobia range from Key West, Florida along the coast to Campeche, Mexico (Dawson 1971). Cobia typically migrate during spring and summer from their wintering grounds off southern Florida to spawning/feeding grounds in the northern Gulf and return to their wintering grounds in late fall and early winter (Biesiot et al. 1994, Franks et al. 1991).

The diet of *R. canadum* from the Gulf of Mexico, particularly the northern Gulf, is poorly known. Most of the previous research on the feeding habits of cobia was limited to simple descriptions of prey items found in a few stomachs. Miles (1949) reported the stomach contents of 11 cobia from Aransas Bay, Texas, and Knapp (1949, 1951) noted the prey found in 24 cobia taken from the same area. Reid (1954), Boschung (1957), and Christmas et al. (1974) commented on feeding in a small number of cobia from Cedar Key, Florida (one fish), coastal Alabama (four fish) and offshore Mississippi (eleven fish), respectively. These researchers found that crustaceans and fish made up the diet of *R. canadum*, although their conclusions varied on the relative importance of each prey type.

Knowledge of the food habits of cobia is necessary for understanding the role of diet in their growth and survival and for comprehending the dynamics of the fishery. The purpose of this study was to describe the diet of cobia from the northcentral Gulf of Mexico.

Materials and Methods

Cobia examined in this study were caught by hook-and-line in the northcentral Gulf recreational fishery from April through October of 1987-1990. Cobia were taken off southeast Louisiana, Mississippi, Alabama, and northwest Florida between lat. 30°25.0'-29°0.0'N and long. 86°0.0'-89°0.0'W. The majority of specimens were taken off coastal Mississippi. Some fish were provided by state and federal fisheries agencies.

Fish were well-iced from the time of capture until stomachs were removed at fishing docks or coastal fishing tournaments. Fork length (FL) was measured in mm and the sex was recorded. Most stomachs were placed in sealable plastic bags and stored in an ice slurry for short-term storage, usually 4-6 h. Stomachs were then either frozen or placed in 10% buffered formalin for later examination. Occasionally, when time permitted, stomachs were removed from fish, opened, and processed in the field.

Stomachs were thawed or removed from formalin, opened, and scored as either containing food or empty. Stomach contents were gently rinsed with fresh water into a 0.5 mm mesh sieve. Prey items were separated, identified to the lowest possible taxon, and counted. Accurate identification and counts could be made in most cases since foods were generally swallowed whole. Some prey items were in advanced stages of digestion and could not be identified to species; however, those prey were often identifiable to the family or order level.

Analyses

All analyses were based on stomachs containing at least one identifiable taxon. Prey too far digested for identification were not used in any computations. Additionally, some items found in stomachs were excluded because they were probably ingested incidentally. Examples of these were tubes of *Chaetopterus* worms, fragments of bivalve and gastropod shells, *Sargassum* weed, and pieces of coral, wood, and leather. Parasitic nematodes and acanthocephalons which occurred in some of the stomachs were also not considered in the diet analyses.

Numeric abundance, frequency of occurrence and percent frequency of occurrence (%F) were tabulated for all identifiable prey. In addition, major prey categories (crustaceans, fish, and cephalopods) were analyzed for percent numeric abundance (%N) and percent frequency of occurrence.

Three different fork length size classes of cobia, small (373-945 mm), medium (950-1,145 mm), and large (1,150-1,530 mm), were selected based on natural breaks within the size frequency distribution, and the percent frequency of occurrence of major prey within each was compared. A contingency table analysis and post-hoc test (Freeman-Tukey transformation) for proportional data were used to determine significant differences (α=0.05) between classes for each major prey category (Zar 1984).

Major prey of male and female cobia were also compared. Since males tended to be smaller than females, only cobia within the size range 590-1,045 mm FL were selected. This range contained most of the males sampled and reduced the confounding effect of size. Tests for significant differences (α=0.05) were made using a Fisher exact test corrected for continuity.

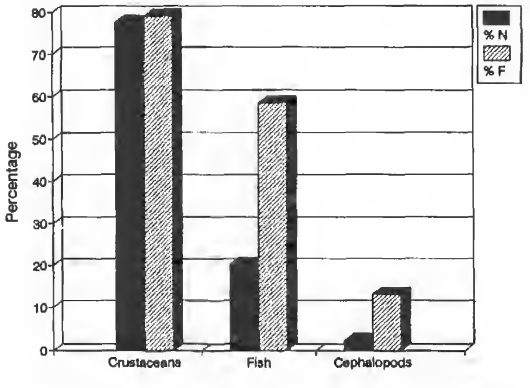

Figure 1. Percent numeric abundance (%N) and percent frequency of occurrence (%F) of major prey categories of *Rachycentron canadum* from the northcentral Gulf of Mexico.

RESULTS

The stomach contents of 403 *R. canadum*, ranging from 373-1,530 mm FL, were examined. Of these stomachs, 287 (71.2%) contained at least one identifiable prey taxon. Prey consisted of crustaceans, fishes, and cephalopods (Table 1). Another 35 (8.7%) stomachs contained only badly decomposed, unidentifiable remains. The remaining 81 stomachs (20.1%) were empty.

Invertebrates

Crustaceans were the primary food of cobia and, essentially, dominated the diet. Crustaceans occurred in 79.1% of the stomachs and ranked first (77.6%) in numeric importance among prey (Figure 1). Crustaceans were represented by eight families of decapods and two families of stomatopods (Table 1).

Portunid crabs were not only the predominant taxa among invertebrates consumed (Table 1) but also represented 60.7%N of total food items in the diet and occurred in 72.8% of the stomachs. The lesser blue crab, *Callinectes similis*, was the most abundant prey species found in the diet, comprising 36.5%N and occurring in 48.8% of the stomachs. The iridescent swimming crab, *Portunus gibbesii*, (12.5%N, 26.5%F) and the ladycrab, *Ovalipes floridanus*, (9.0%N, 23.3%F) were the next most important foods in the diet.

Following the portunids in importance were the sicyoniids and penaeids (combined=9.6%N). Other decapods, *i.e.*, callianassids, calappids, majids, pagurids and xanthids, occurred infrequently (Table 1). Stomatopods, predominantly Squillidae, comprised 6.9%N of the diet.

Cephalopods comprised the other primary invertebrate prey group and were represented by two families, Loliginidae, the predominant group, and Octopodidae. Cephalopods were found in 13.2% of the stomachs but only made up 2.2%N of prey consumed (Figure 1).

TABLE 1

Prey items occurring in stomachs of cobia, *Rachycentron canadum*, from the northcentral Gulf of Mexico, 1987-90. Percent frequency of occurrence based on *N*=287.

Prey	Total number of individual prey items	Frequency of occurrence	Percent frequency of occurrence
INVERTEBRATES			
Crustaceans			
Decapoda			
Penaeidae			
Penaeus aztecus	3	1	0.3
Penaeus setiferus	1	1	0.3
Penaeus sp.	34	8	2.8
Trachypenaeus sp.	37	9	3.1
Sicyoniidae			
Sicyonia brevirostris	62	15	5.2
Sicyonia sp.	102	18	6.3
Callianassidae			
Callichirus islagrande	1	1	0.3
Paguridae sp.	2	2	0.7
Calappidae			
Calappa flammea	2	1	0.3
Hepatus epheliticus	2	2	0.7
Majidae			
Libinia emarginata	1	1	0.3
Portunidae			
Arenaeus cribrarius	16	8	2.8
Callinectes sapidus	5	5	1.7
Callinectes similis	909	140	48.8
Ovalipes floridanus	224	67	23.3
Portunus gibbesii	312	76	26.5
Portunus sayi	1	1	0.3
Portunus spinicarpus	16	3	1.0
Portunus spinimanus	30	17	5.9
Xanthidae			
Menippe adina	1	1	0.3
Stomatopoda			
Lysiosquillidae			
Lysiosquilla scabricauda	2	2	0.7
Squillidae			
Squilla chydaea	2	2	0.7
Squilla empusa	78	21	7.3
Squilla neglecta	1	1	0.3
Squilla sp.	88	40	13.9
Cephalopods			
Loliginidae			
Loligo pealei	1	1	0.3
Unid. loliginids	47	33	11.5
Octopodidae			
Octopus sp.	6	4	1.4
FISH			
Squatinidae			
Squatina dumeril	1	1	0.3
Dasyatidae			
Dasyatis sp.	7	7	2.4
Torpedinidae			
Narcine brasiliensis	4	3	1.0
Anguilliformes	133	52	18.1

Prey	Total number of individual prey items	Frequency of occurrence	Percent frequency of occurrence
Clupeidae			
Brevoortia patronus	19	3	1.0
Brevoortia sp.	2	2	0.7
Unid. clupeids	4	4	1.4
Engraulidae			
Anchoa sp.	2	1	0.3
Unid. engraulid	1	1	0.3
Ariidae			
Arius felis	138	70	24.4
Ophidiidae	5	4	1.4
Ogcocephalidae			
Halieutichthys aculeatus	1	1	0.3
Syngnathidae	2	2	0.7
Triglidae			
Prionotus sp.	48	7	2.4
Serranidae			
Diplectrum bivitatum	33	2	0.7
Unid. serranids	2	1	0.3
Carangidae			
Decapterus punctatus	26	18	6.3
Seriola dumerili	1	1	0.3
Unid. carangid	1	1	0.3
Lutjanidae			
Lutjanus campechanus	3	3	1.0
Sparidae			
Lagodon rhomboides	10	10	3.5
Unid. sparid	1	1	0.3
Sciaenidae			
Menticirrhus sp.	3	3	1.0
Micropogonias undulatus	9	3	1.0
Cynoscion sp.	1	1	0.3
Leiostomus xanthurus	1	1	0.3
Mugilidae			
Mugil sp.	5	3	1.0
Uranoscopidae			
Astroscopus y-graecum	5	5	1.7
Trichiuridae			
Trichiurus lepturus	3	1	0.3
Stromateidae			
Peprilus burti	1	1	0.3
Peprilus sp.	3	1	0.3
Bothidae			
Citharichthys sp.	12	3	1.0
Etropus crossotus	1	1	0.3
Etropus sp.	2	1	0.3
Soleidae			
Symphurus plagiusa	1	1	0.3
Symphurus sp.	1	1	0.3
Balistidae			
Balistes capriscus	1	1	0.3
Unid. balistids	4	3	1.0
Tetraodontidae			
Chilomycterus schoepfi	2	2	0.7
Unid. tetraodontids	6	3	1.0
	Total 2,491		

Number of stomachs examined	403
Number (and %) of stomachs containing identifiable prey	287 (71.2)
Number (and %) of stomachs containing only decomposed, unidentifiable remains	35 (8.7)
Number (and %) of empty stomachs	81 (20.1)

Fish

Although contributing substantially to the diversity of the diet, fish were not as important as crustaceans. Fish occurred in 58.5% of the stomachs and accounted for 20.3%N of all prey consumed (Figure 1). A wide variety of fishes was consumed, including twenty families of bony fishes and three families of cartilaginous fishes (Table 1).

The hardhead catfish, *Arius felis*, and eels (Order Anguilliformes) were by far the predominant fishes in the diet. *Arius felis*, found in 24.4% of stomachs, exhibited the highest numeric percentage (27.3%) among fish and contributed 5.5%N to the total diet. Eels occurred in 18.1% of stomachs, comprised 26.3%N of fish in the diet, and accounted for 5.3%N of total items in the diet.

Fish less frequently encountered in the diet included round scad, *Decapterus punctatus* (Carangidae) and pinfish, *Lagodon rhomboides* (Sparidae). Other identified fish occurred only rarely (Table 1).

Comparison of diet among size classes of cobia

Crustaceans dominated the diet of the small (77.2%F) and medium (84.8%F) size classes of cobia, and made up a primary portion (65.6%F) of the large size class (Figure 2). Despite these high frequencies, contingency table analysis (χ^2=10.25, df=2, p<0.05) and the corresponding post-hoc tests indicated all three size classes were significantly different from each other. Portunid crabs, particularly *Callinectes similis*, were the most important prey consumed in all size classes of cobia (Table 2).

In contrast, the importance of fish as prey increased with increasing size of cobia, the largest size class showing the highest percent frequency of occurrence (84.4%) (Figure 2). The increase in fish occurrence was attributable to the hard-head catfish, *Arius felis*, which increased from 7.0%F in the small size class to 43.8%F in the large cobia (Table 2). Again, contingency table analysis (χ^2=27.77, df=2, p<0.001) and post-hoc tests indicated that all size classes were significantly different from each other.

The percentage of cephalopods (predominantly squid) remained consistently low across the three size classes (Figure 2, Table 2). No significant differences were found.

Comparison of the diets of male and female cobia

The diet of male and female cobia within the size range of 590-1,045 mm FL appeared to be similar (Table 3). Crustaceans were the dominant prey in both sexes. Although females showed a higher percent frequency of occurrence (86.8%) of crustaceans than did males (79.2%), these differences were not significant. Portunid crabs were the major component of crustaceans ingested by both sexes.

Fish occurred with greater frequency in the diet of males (60.4%F) than in the diet of females (46.2%F), partially due to a greater occurrence of eels in the male diet (Table 3). Males, however, fed less frequently on catfish. As with the crustacean prey, no significant differences were found between the diets of male and female cobia with respect to fish or cephalopod prey.

TABLE 2

Percent frequency of occurrence of major taxa in the stomachs of three size classes of *Rachycentron canadum* from the northcentral Gulf of Mexico.

Fork length (mm)	373-945	950-1145	1150-1530
	N=57	*N*=164	*N*=64
Crustaceans			
(Portunid crabs)	(63.2)	(80.5)	(64.1)
Callinectes similis	35.1	53.0	51.6
Portunus gibbesii	17.5	31.1	23.4
Ovalipes floridanus	19.3	28.0	15.6
Stomatopods	24.6	19.5	25.0
Fish			
Anguilliformes	14.0	19.5	18.8
Arius felis	7.0	22.0	43.8
Cephalopods			
Loliginidae	17.5	9.1	14.1

TABLE 3

Percent frequency of occurrence of major taxa from the stomachs of male and female *Rachycentron canadum* from the northcentral Gulf of Mexico. Size range from 590-1045 mm FL.

	Male	Female
	N=48	*N*=106
Crustaceans		
(All Crustaceans)	(79.2)	(86.8)
(Portunid crabs)	(70.8)	(80.2)
Callinectes similis	33.3	52.8
Portunus gibbesii	20.8	32.1
Ovalipes floridanus	16.7	30.2
Stomatopods	14.6	19.8
Fish		
(All Fish)	(60.4)	(46.2)
Anguilliformes	27.1	17.9
Arius felis	6.3	17.0
Cephalopods		
Loliginidae	10.4	14.2

DISCUSSION

We found crustaceans, primarily portunid crabs, to be the dominant foods of cobia both in terms of numeric abundance and percent frequency of occurrence. Fishes were second in order of importance. These results vary somewhat from the findings of other researchers. Miles (1949) reported crabs, shrimps, and fishes in near equal numbers in the stomachs of cobia taken from Aransas Bay, Texas, and, similarly, Christmas et al. (1974) found the numbers of fishes and crustaceans to be approximately the same in their samples from northern Gulf waters off Mississippi. In sharp contrast, Knapp (1951) observed a predominance of fishes (83.3%F), followed by stomatopods (58%F), penaeid shrimps (46%F) and crabs (42%F) in the diet of cobia caught near Aransas Bay, Texas. The conclusions reached in previous studies were based on examinations of a limited number (24 or less) of stomachs. Although cobia examined in our study were collected by hook-and-line and, therefore, did not represent a random sample, we believe our findings represent a more definitive description of the diet of cobia in the northern Gulf of Mexico, due, in part, to our high sample number (N=287) and extensive geographical range.

Although crustaceans were the dominant food, our results also indicated that larger cobia, males and females alike, consumed fish with significantly greater frequency than did smaller cobia. This may reflect an ontogenetic shift toward fish as prey in larger cobia. Our results, however, showed no significant differences in the diet of male and female cobia within a range of comparable sizes which may be attributable to the relatively low sample size of males. Although not statistically different, we did encounter fish more frequently in the stomachs of males than females which also may be an indication of an ontogenetic shift toward fish prey since most of the large males, and not the large females, were included in the male-female comparative analysis.

The species composition of the diet revealed that cobia fed primarily on or near the sea floor. The portunids, sicyoniids, penaeids, and stomatopods, though capable of swimming, are primarily benthic or epibenthic inhabitants. Octopi, as well as many of the fish prey (*e.g.*, bothids, uranoscopids, arrids, triglids, dasyatids, eels), also reside on or near the bottom. However, other prey such as carangids, clupeids, and squid are pelagic organisms, and their presence in the diet indicated flexibility in the foraging behavior of cobia.

In summary, we found that the primary foods of cobia from the northcentral Gulf of Mexico were benthic or epibenthic crustaceans and fishes, although some feeding did occur in the water column and nearsurface. Additionally, our results indicate that the cobia is an opportunistic carnivore and that feeding appears to depend more on prey availability rather than upon a few specific food organisms.

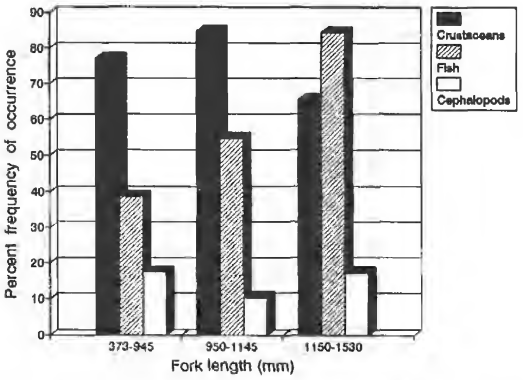

Figure 2. Percent frequency of occurrence of major prey categories for three size classes of *Rachycentron canadum* from the northcentral Gulf of Mexico.

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We thank Tom McIlwain for his advocacy of this work. We would also like to thank coastal sportfishermen and the directors of coastal fishing tournaments in Mississippi and Florida for their spirit of cooperation in helping us obtain samples. We are grateful to Richard Heard and Bruce Comyns for their help in identifying the invertebrates and the fishes, respectively. The field assistance provided by Carol Cleveland, Mike Buchanan and T.J. Becker was greatly appreciated. We give our thanks to Terry McBee for his help in the field and for his valuable assistance with data analysis. Our gratitude is extended to Barbara Viskup and David Aborn for reviewing an earlier version of the manuscript. Jeffery Lotz also provided helpful suggestions which improved the manuscript. The constructive comments of two anonymous reviewers were also appreciated. We thank marine enforcement personnel of the Mississippi Dept. of Wildlife, Fisheries and Parks for providing us with some specimens of cobia. Funding for this study was provided by the U.S. Dept. of the Interior, U.S. Fish and Wildlife Service, Federal Aid in Sportfish Restoration Project F-91, through the Mississippi Dept. of Marine Resources.

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Seasonal Migration in the Southern Hogchoker, *Trinectes maculatus fasciatus* (Achiridae)

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SEASONAL MIGRATION IN THE SOUTHERN HOGCHOKER, *TRINECTES MACULATUS FASCIATUS* (ACHIRIDAE)

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ABSTRACT Life history patterns often respond to local environmental conditions. The seasonal migration pattern of the northern hogchoker has been described, but the southern subspecies rarely has been studied. To document the migratory movements and the habitat characteristics of the southern hogchoker, long-term survey data and specimens collected during 1993 were examined. Moderate depth (5.8-6.4 m), low water clarity (0-1.2 m), moderate oxygen concentration (4-9 ppm), and sand-mud substrata generally defined hogchoker habitats. Hogchoker habitats only showed seasonal shifts in temperature and salinity characteristics. Hogchokers were only collected in low salinity (0-2 ppt) waters during the winter, but exhibited three abundance peaks in relation to bottom salinity during the summer samples at 0, 5, and 18 ppt. The survey data and the data from the 1993 specimens support the hypothesis that southern hogchokers are following a migration pattern similar to that described for the northern subspecies.

INTRODUCTION

The distribution of *Trinectes maculatus fasciatus*, the southern hogchoker, extends south from approximately South Carolina to the Yucatán peninsula. The range of the northern subspecies (*T. m. maculatus*) extends from the South Carolina coast north to Massachusetts (Hildebrand and Cable 1938, Gilbert and Kelso 1971). Hogchokers are small estuarine fish with a complicated migration pattern. Newly-hatched individuals begin moving into freshwater areas following summer estuarine spawning and begin migrating into low salinity areas the next spring. This downstream distance is extended progressively each year until maturity, when spawning occurs in the outer areas of the estuary. A return migration into freshwater occurs each fall for the winter period (Dovel et al. 1969).

Life history patterns may vary in response to local ecological conditions and the timing of environmental factors can often dictate differences in the evolution of these traits (Stearns 1976, Boyce 1979). The migratory movements and many life history factors of the northern hogchoker have been widely studied. However, the southern subspecies has only been the subject of a few studies along the Atlantic coast (Castagna 1955, Smith 1986).

The purpose of this study was to determine if the movement pattern documented in the northern subspecies is also present in a Gulf population of southern hogchokers. While individual collections have documented hogchokers in both freshwater streams and estuaries along the Mississippi coast, I also examined continuous survey data to clarify seasonal movement patterns. These survey data were also used to describe habitat characteristics of *T. m. fasciatus*. Finer details of the migration pattern were investigated by examining the reproductive condition and age of hogchokers along the salinity gradient during 1993.

MATERIALS AND METHODS

Hogchoker distribution and habitat data were obtained from a fishery survey conducted since 1980 along the salinity gradient from the Back Bay of Biloxi offshore to Horn Island by personnel of the Gulf Coast Research Laboratory (GCRL), Ocean Springs, Mississippi. The survey samples consisted of standardized 10 minute tows with a 4.9 m flat otter trawl. Deeper trawls, at stations 83 and 84, required 30 minute tows with a 12.2 m flat otter trawl. Both trawls consisted of a 19.1 mm stretch mesh body with a 6.4 mm mesh cod end liner. Monthly collections were made at six sites along the salinity gradient and hogchokers were commonly caught at four of these sites: Bayou Bernard (36), Keesler Marina (34), Biloxi East Channel (37), and Bellefountain Buoy 8 (32) (Figure 1).

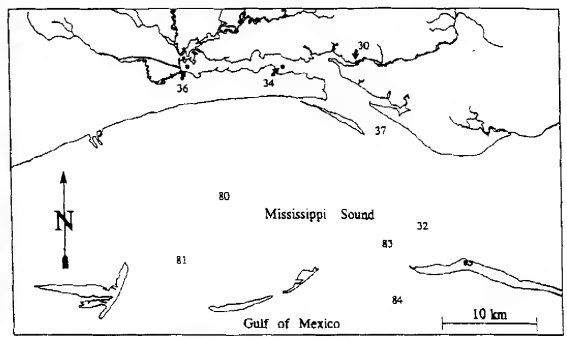

Figure 1. Distribution of sampling localities for the GCRL survey in the Back Bay of Biloxi, Mississippi.

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Hogchoker abundance, bottom temperature, bottom salinity, bottom oxygen concentration, water clarity (Secchi disk) and several habitat classifications (water body, bottom morphology, and substratum) were measured at each site. A factor analysis ($SPSS_x$-V2.1) of all environmental variables was performed to specifically describe hogchoker habitats. Although the assumptions were validated, this procedure was abandoned because even if all 11 variables were included, only 40% of the variance in hogchoker abundance could be explained. This variability is probably attributable to the complicated and interactive nature of the factors associated with the hogchoker's seasonal movements along the salinity gradient. Instead, frequency distributions of the number of hogchokers collected during the survey were examined for each variable. In examining the trends, the presence of 45 individuals was considered to be biologically meaningful. The data were examined in three sets: the entire sample, a summer sample (May through August), and a winter sample (October through March). When no differences occurred between the seasonal data sets, only the entire data set is presented.

To address reproductive condition, the specimens collected during the 1993 monthly survey trawls were examined. Two additional stations, 80 and 81, were sampled in the Mississippi Sound during June and July 1993 using 12.2 m trawls. A low salinity site (station 30), near the mouth of Old Fort Bayou, was also sampled in May and September 1993 with 3.2 mm mesh seines.

All *T. maculatus* were fixed in 10% seawater formalin. Each specimen was weighed (0.1 mg), measured (SL and TL, 0.01 mm) and then dissected to remove the gonads and otoliths. Gender was determined and reproductive condition of females was classified following Smith (1986) (Table 1). Gonadosomatic indices (GSI) (Nielson and Johnson 1983) were calculated for each specimen as the wet gonad weight divided by whole wet body weight multiplied by 100. The assumptions of a linear relationship and a 0 y-intercept for a gonad and body weight regression were validated before using the GSI. The average GSI values were compared between stations.

The otoliths were embedded in Ciba Geigy® media and sectioned with a Beuhler Isomet® Low Speed Saw into 1.0-2.0 mm increments. The sections were hand ground to a 0.20-0.50 mm thickness using 600 and 1500 grit sandpaper and then polished (Beuhler® Alpha Micropolish II). The otoliths were aged by three independent readers and only used if at least two of the three readings agreed. Symmetrical growth between otoliths and the annular formation of rings were validated in Peterson (in preparation). Age distributions were compared between stations along the salinity gradient.

TABLE 1

Female reproductive classifications based on external morphology of the ovaries, following Smith.

Category	Color	Shape	Tissue
Immature	pale	small, equilateral triangle; no posterior elongation	undifferentiated; no vascularization
Resting	light yellow	more robust; some posterior elongation (length = 2X height)	folicular development; no vascularization
Developing	deep yellow	more elongated posteriorly; becoming turgid and distended	appears granular from folicular development; slight vascularization
Ripe	dark yellow to orange	extended to the distal end of the coelomic cavity; very distended	appears granular with distinct eggs visible; highly vascularized
Spent	pale to yellow; often with a reddish hue	elongated but extremely flacid; appearing deflated	folicular material loose, with atretic eggs present; vascularization disrupted

RESULTS

A total of 936 collections were made from 1980-1993. The salinity gradient from Biloxi Bay out to Horn Island was stable, but there was substantial yearly variation in the absolute salinities (Figure 2).

Hogchoker abundance was not seasonally associated with changes in water depth, water clarity or oxygen concentrations. Hogchokers occupied depths from 2.4-6.7 m, with most captured in 5.8-6.4 m of water (Figure 3a). The available habitat range extended from 0.6 to 11.6 m. The water clarity of the sample area usually ranged from 0.0-2.4 m, with a few samples reported from 3.1-7.6 m. Hogchokers were mostly associated with habitats of 0.0-1.2 m of visibility (Figure 3b). The Back Bay of Biloxi had oxygen concentrations ranging from 4-12 ppm over the study area. The highest abundance of hogchokers were collected in habitats with 4-9 ppm oxygen (Figure 3c).

There were definite seasonal trends, as expected, in the temperature of habitats utilized by hogchokers. Throughout the sample period, temperatures ranged from 1-36°C. During summer months, hogchokers were most abundant in temperatures of 24-32°C; while during the winter peak abundance occurred between 12 and 19°C (Figure 4).

Hogchokers also did not exhibit seasonal shifts in the use of habitat types. They were usually in the Bay and Bayou, sometimes collected in the Sound, and only rarely in the Gulf (Figure 5a). They were also commonly collected in natural and dredged channels, but less so in open water without submerged vegetation (Figure 5b).

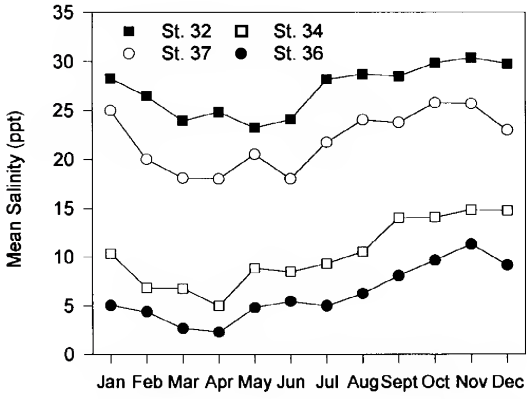

Figure 2. Monthly average salinity levels of four sampling stations in the Back Bay of Biloxi from a 14-year survey. 95% confidence intervals were large and, for clarity, are presented in Peterson (1994).

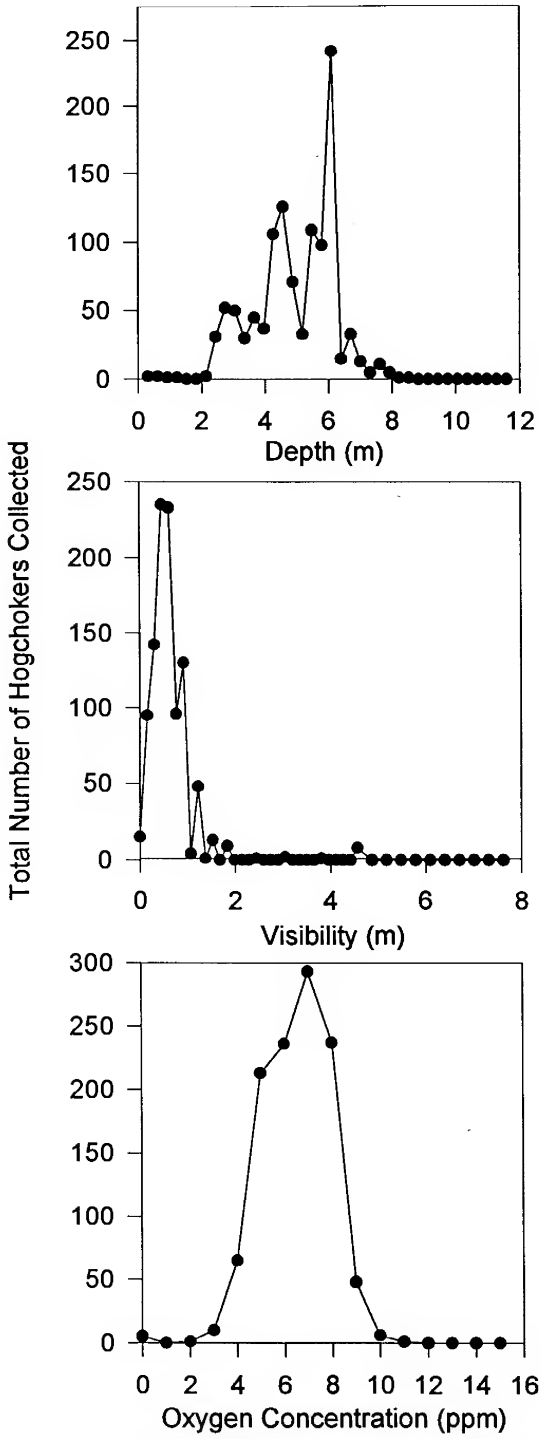

Figure 3. Hogchoker abundance distribution in relation to habitat depth, water clarity and bottom oxygen concentration.

In addition to these categories, only three others (sand beaches with submerged vegetation and marsh areas both with and without submerged vegetation) were sampled, a total of 11 times, throughout the sample period. Hogchokers were documented in each of these habitat types, but they were most abundant in habitats with mud bottoms (Figure 5c). However, during the study there were no sites classified as sand substrata, probably indicating an actual sand-mud combination along the salinity gradient.

Hogchokers were collected during all months, but they were most abundant from April to September (Figure 6a). Abundance also varied among years, with a peak from 1988-1989 (Figure 6b). This peak is followed by a sharp decline in collection abundance, with a subsequent increase in 1993.

Hogchokers did exhibit a seasonal shift in terms of habitat salinity. During the winter, hogchokers were most abundant in salinities of 0-2 ppt (Figure 7), with only rare occurrences in higher salinity areas. During the summer, three abundance peaks occurred, at approximately 0, 5, and 18 ppt. Hogchokers were documented throughout most of this salinity range during summer collections.

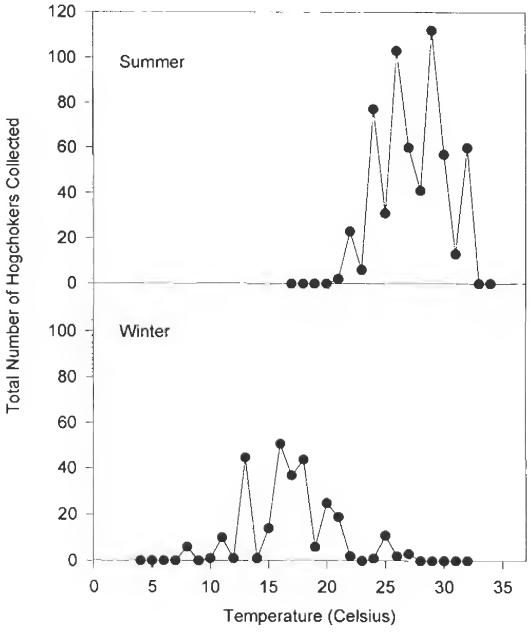

Figure 4. Hogchoker abundance distribution in relation to bottom temperature during summer and winter sample periods.

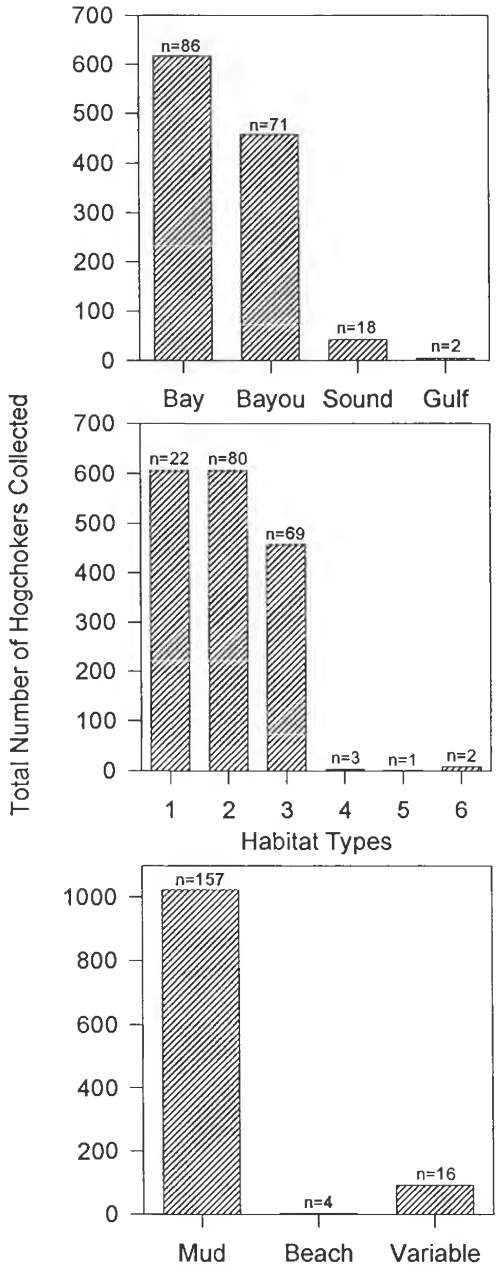

Figure 5. Distribution of hogchokers among categories of water body types, bottom morphology (Coding: 1-open water without submerged vegetation, 2-dredged channel, 3-natural channel, 4-sand beach without submerged vegetation, 5-marsh with submerged vegetation, 6-marsh without submerged vegetation), and substrata characteristics. Sample n's are the number of collections with hogchokers in each category.

In 1993, salinity generally increased along the gradient (Figure 8). Hogchokers were collected during April only at lower salinity stations. During June, most hogchokers were collected at the outer and higher salinity stations. Hogchokers were again only collected in low salinity areas in September. The salinity ranges during January-July 1993 were similar to the survey data means.

Immature individuals, males, and resting and developing females were collected in April. In June, only resting females were collected in low salinities, while ripe and developing females were collected at the Sound stations. Ripe females were only collected during June at the high salinity stations. Hogchokers, including a spent female, were only collected in July at a low salinity station in the bay. Only immature specimens were collected at the mouth of Old Fort Bayou during September (Figure 9).

The gonadosomatic indices were low (< 0.5) for all specimens collected in April and male GSI values never exceeded 0.5 in the 1993 samples. However, female GSI values increased to 5.0-6.1 at the outer stations during June, and then decreased in the next month (Figure 10).

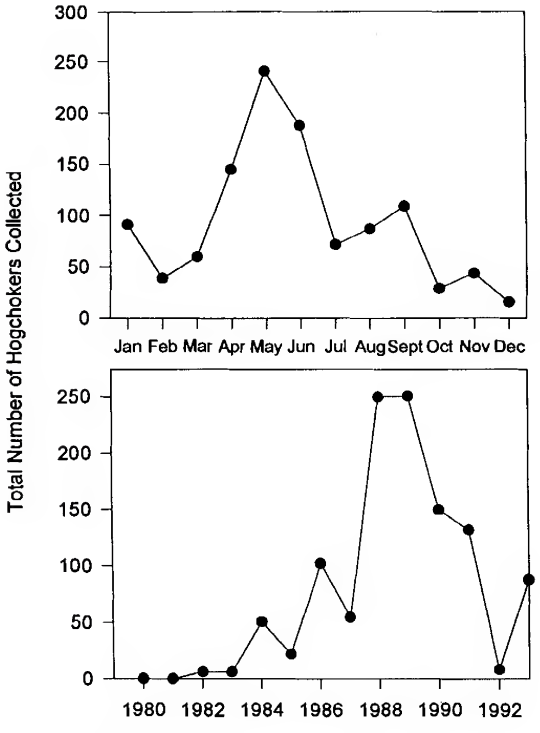

Figure 6. Total collection abundance of hogchokers during each month and year of the survey.

During the summer months in which hogchokers were collected, a trend of increasing age along the salinity gradient was exhibited (Figure 11). Young of the year fish were only collected at the low salinity sites and the oldest specimen, in its fifth summer, was collected at the outermost station. On the spawning grounds, considered to be stations 80 and 81 based on the reproductive data, the mean age of hogchokers was approximately 3 to 4 years.

DISCUSSION

The annual pattern of hogchoker abundance could be indicating population trends. If so, the Biloxi population of southern hogchokers increased in size from 1984 to 1989, followed by a sharp population decline in the following years and only in 1993 did an upward trend return. This could be the result of variation in annual recruitment and survival. To my knowledge, no major environmental phenomena occurred which could explain this pattern and annual salinity fluctuations do not correspond with the population trends. Further investigations of hogchoker population dynamics are needed to explain this annual variation.

Hogchoker habitats can be described as areas of moderate depth, low water clarity, moderate oxygen concentration, and mud-sand substrata. Temperature and

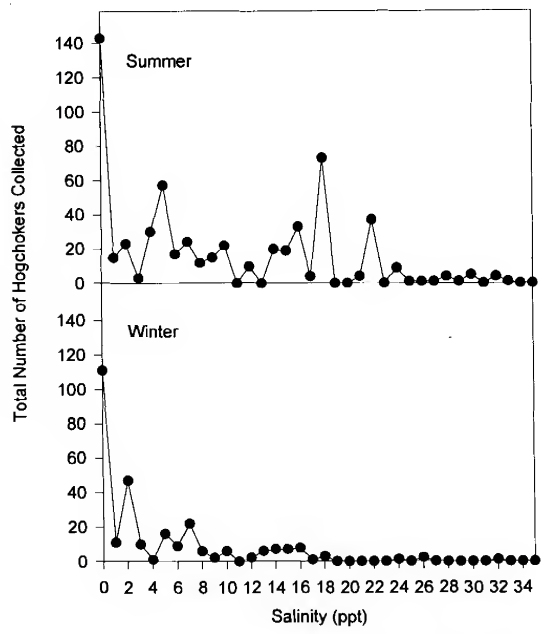

Figure 7. Distribution of hogchokers in relation to bottom salinity during summer and winter sample periods.

salinity were the only variables in which hogchokers showed a seasonal shift in resource use. These results correspond with Smith's (1986) suggestion that temperature initiates the migration while salinity is the directing factor. During winter months, hogchokers were most common in low salinity waters of 12-19°C. During the summer, there were three main hogchoker locations, all of 24-32°C. One area was at the freshwater interface, another at 5 ppt, and the final locality at 18 ppt.

The 1993 collections exhibited abundance trends similar to the survey data. Hogchokers were first collected in April only in low salinity areas, their GSI values were low and none of the specimens were mature. As summer progressed, the older maturing and mature fish began moving out into the estuary. The abundance peak in the survey data at the freshwater interface were most likely immature individuals, and 2-3 year old maturing specimens probably represent the 5 ppt peak (Peterson in prep.). Peters and Boyd (1972) reported a similar summer distribution of juvenile hogchokers in a North Carolina population, with the highest abundance occurring at 25°C and 5-10 ppt. The final peak at 18 ppt represents the hogchoker spawning grounds. The only specimens seen in spawning condition, as well as the oldest individuals, were collected in this area. The distribution range then shortens as the fish move back to low salinity and freshwater habitats. This would explain why the winter survey samples produced only a single abundance peak in low salinity (0-2 ppt) areas.

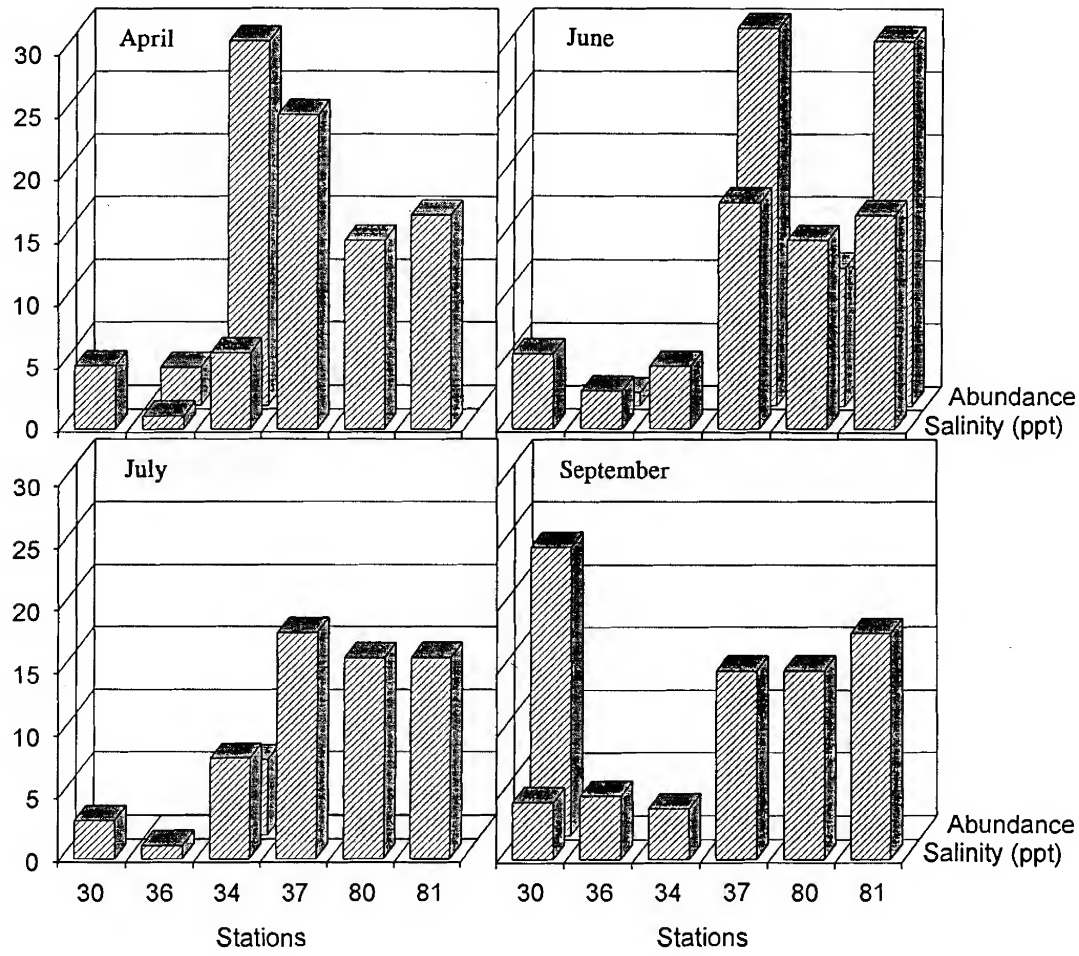

Figure 8. Habitat salinity and hogchoker abundance at each station along the salinity gradient during 1993.

Dovel et al. (1969) reported that hogchoker spawning areas are in full seawater (30ppt). The data presented here suggest that southern hogchokers are spawning in lower salinity waters, approximately 15-18 ppt. This may not represent a true difference, but instead may be an artifact of environmental characteristics. The northern Gulf of Mexico does not have the higher salinity inshore areas as those reported for the northern Atlantic coast. Moreover, the spawning area reported by Dovel et al. (1969) was determined by egg presence in plankton samples, not the presence of ripe hogchokers. Further studies of the northern subspecies, or hogchoker egg collections in the Gulf of Mexico, are required to verify this possible subspecies difference.

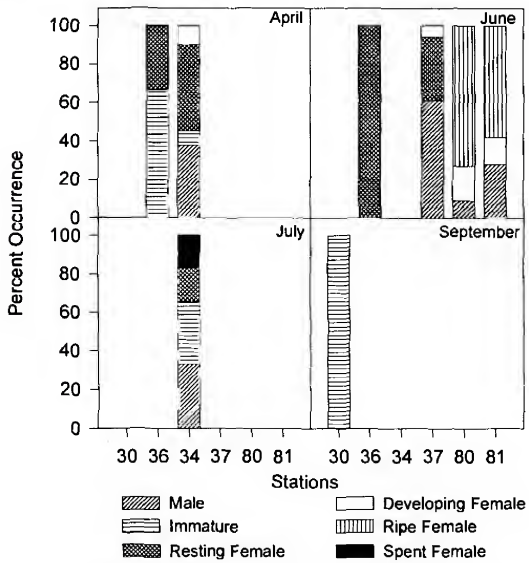

Figure 9. Percent occurrence of gender and female reproductive conditions of hogchokers collected at each station along the salinity gradient during 1993.

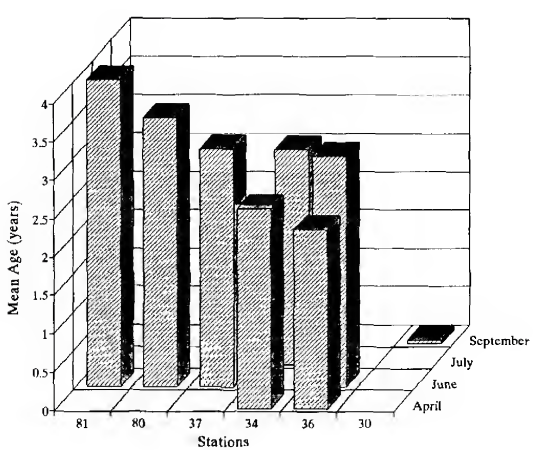

Figure 11. Mean age of hogchokers collected at each station along the salinity gradient during 1993.

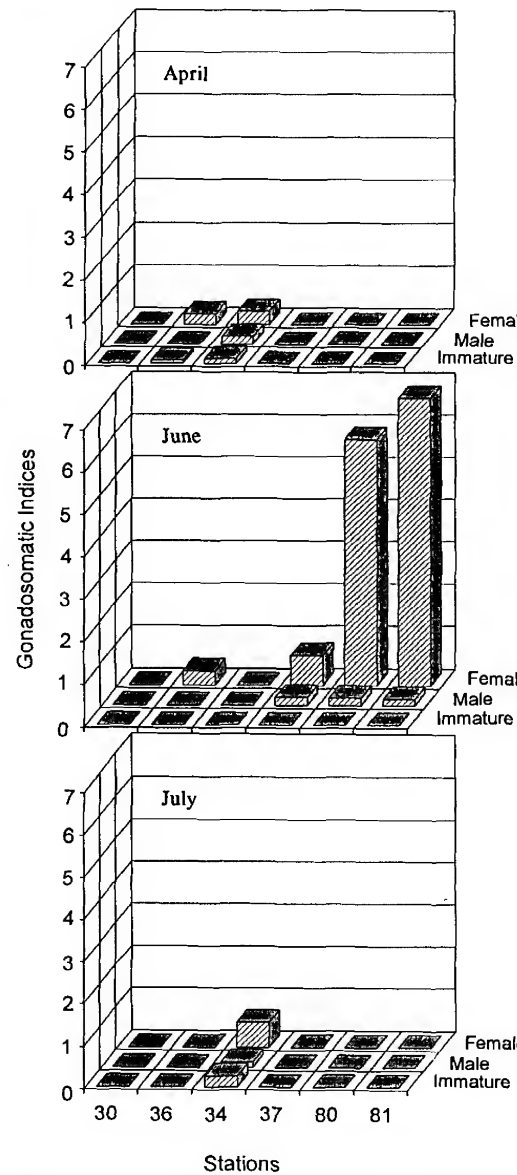

Figure 10. Mean gonadosomatic indices of hogchokers collected at each station along the salinity gradient during 1993.

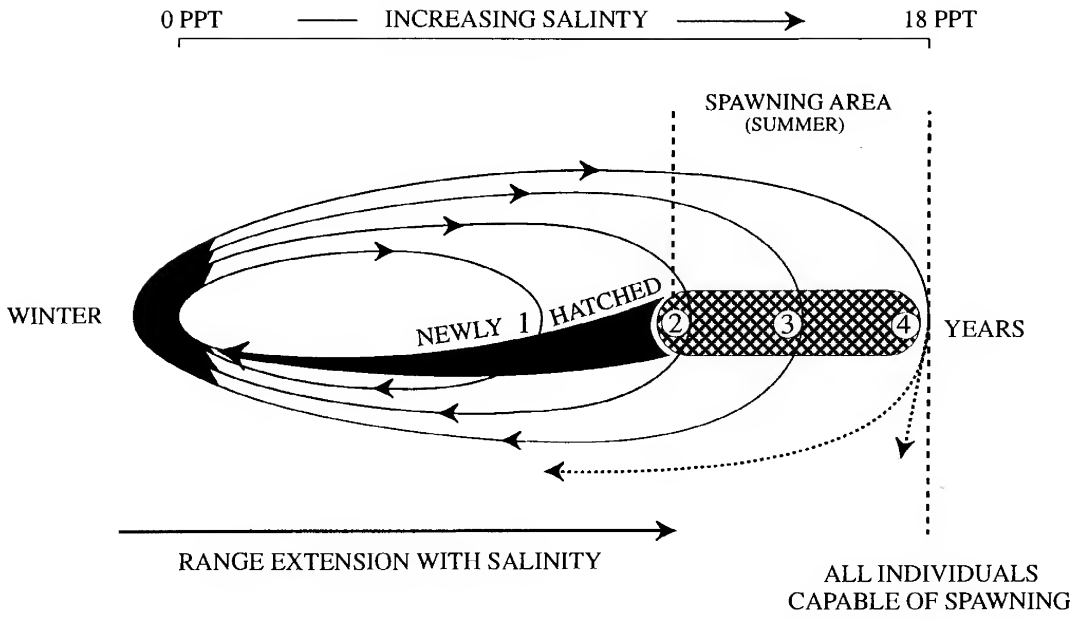

Figure 12. Proposed seasonal migration pattern of southern hogchokers, modified from Dovel et al. (1969).

The survey data and the 1993 movement patterns support the hypothesis that the Back Bay of Biloxi population of southern hogchokers uses a migration pattern similar to that described for the northern subspecies. A migration pattern, modified from Dovel et al. (1969), is presented as that followed by the southern subspecies (Figure 12). The conservation of this character between subspecies and over time agrees with McDowell's (1993) suggestion that diadromy is a stable process and not a transitional mechanism of evolving to a freshwater lifestyle.

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Reproductive Strategies in a Population of *Gobiosoma bosci* (Osteichthyes: Gobiidae) with Slow and Fast Maturing Individuals

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REPRODUCTIVE STRATEGIES IN A POPULATION OF *GOBIOSOMA BOSCI* (OSTEICHTHYES: GOBIIDAE) WITH SLOW AND FAST MATURING INDIVIDUALS

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ABSTRACT The reproductive biology of *Gobiosoma bosci* collected from November 1986 to October 1987 in the McFaddin Wildlife Refuge in southeast Texas was studied by using morphometric data. Males achieved greater weights per unit length than females, and longevity was about 12 to 13 months. GSI values and mean monthly ovum diameters indicated that the breeding season ran from April to September, with a major activity peak in May and a minor peak in September. Significant differences in male and female standard lengths (SL), ovum diameter, and egg number existed for sexually mature specimens between the first and second peaks of reproductive activity. An egg versus length analysis produced a positive linear relationship. An accessory gonadal structure index (ASGI) was developed and revealed that maximal AGS development corresponded with the male GSI, but did not produce discernable peaks. Two reproductive strategies were followed and depended upon time of hatching and growth rate. Some individuals that hatched early in the breeding season grew rapidly and were capable of egg laying by August or September. Individuals hatched late in the breeding season delayed breeding until the following season.

Introduction

The distribution of the naked goby, *Gobiosoma bosci*, extends from Long Island, New York, to the state of Campeche, Mexico (Hoese and Moore 1977). In view of the marked environmental differences throughout its range, *G. bosci* may be expected to vary its reproductive strategies. In a review of life history phenomena, Cole (1954) stated that the age at which reproduction begins is one of the most significant characteristics, and as such will influence the reproductive success of an individual. Therefore, southerly populations of *G. bosci* have at least two potential reproductive strategies available to them (Stearns and Crandall 1984). First, an individual may grow throughout the non-reproductive season after hatching and then engage in breeding during the subsequent breeding season. Alternatively, young-of-the-year may grow rapidly enough to reproduce before the end of the breeding season, and thereby gain a breeding season not available to fish with delayed breeding. As such, individuals within the same population may employ one strategy or the other depending upon their time of hatching and environmental conditions.

Gobiosoma bosci, a cavity nester preferring hard substrates (Bechler et al. 1990), has been studied extensively along the Atlantic Coast. Dahlberg and Conyers (1973), who reviewed much of the literature, postulated that spawning seasons for different populations of *G. bosci* were variable, and depended on location. They related initiation and termination of spawning to water temperature and indicated peak spawning activity was in the warmest months, May through August. However, they did not discuss any variations in breeding strategies. Other reports on *G. bosci* by Nero (1976), Crabtree and Middaugh (1982), and Fitzsimons and Seok (1989) revealed many facts about the life history and ecology of *G. bosci*. However, none of the above studies examined reproductive strategies of the naked goby in detail along the Gulf Coast. In this study, we concentrated specifically on life history data related to the reproductive biology of *G. bosci* in a southeast Texas salt marsh.

Description of the Study Area

The McFaddin National Wildlife Refuge is located in southern Jefferson County, 20 km southwest of Sabine Pass, Texas (Griffith and Bechler 1995), a subtropical to temperate region of the United States. The refuge is a brackish marsh dissected by meandering creeks, man-made cuts, and shallow lakes; the largest, Clam Lake, was the primary site of this study.

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Clam Lake is relatively shallow, 1.0 to 1.3 m in depth, with the deepest area 2.8 m. The substrate and banks along the edges of the lake consist of a firm clay. A fine silt substrate lies 1 to 5 m from shore. The clay banks are riddled with holes and tunnels inhabited by *Rhithropanopeus harrisi*, the mud crab, two species of *Uca*, the fiddler crabs, and *Callinectes sapidus*, the blue crab. The predominant flora surrounding the lake is *Phragmites australis*, a reed grass, and *Spartina alterniflora*, a salt marsh grass. Tidal influences in Clam Lake are minimal. Wind-generated wave action undercuts the banks of the lake and results in the gradual erosion of the shoreline and a subsequent exposure of roots of *Phragmites* and *Spartina*. These roots are washed free of soil and extend into the water except during drought and low tide. Large clumps of *Phragmites* roots break off and fall into the water. These remained close to the banks of the lake below the water and provided habitat for *G. bosci*.

METHODS AND MATERIALS

Beginning in November 1986, monthly collections of *G. bosci* were made in Clam Lake for a period of 12 months, except for July, when no collection was made. Sixty to 100 fish were collected each month by using a 3.23 mm mesh seine. Specimens were hardened in 10% formalin for 24 hours, washed in water for 24 hours, and preserved in 55% isopropyl alcohol. A minimum of 28 specimens (14 males, 14 females) were examined from each monthly collection. Standard length (SL) was measured to the nearest 0.01 mm with a Mitutoyo dial caliper. Total body weight was measured to the nearest 0.0001 g with a Mettler AE 100 analytical balance. Gonads were removed and wet weighed together as a unit for use in obtaining Gonadal Somatic Indices (GSI). Ten accessory gonadal structures (AGS), which are related to sexual maturation of male gobies (Miller 1984, Cole and Robertson, 1988, Lahnsteiner et al. 1992), were removed and wet weighed each month. The weight of the AGS was divided by the total wet body weight and multiplied by 100 to produce an Accessory Gonadal Structure Index (AGSI) similar to that of the GSI. In females, 10 ova centrally located on the surface of the ovary and randomly selected were measured in 10 animals each month with an ocular micrometer. During the months of April and May, the beginning of the breeding season, the diameters of 175 to 200 ova on the surface of the ovaries were measured in six fish to determine if more than one size class of ova were present. Eggs large enough to be teased from the ovaries were counted by hand with an Olympus zoom stereo microscope system. These ova were classified as to their developmental stage by following Heins and Rabito (1986). Salinity and water temperature were taken each month with a Yellow Springs TSC meter. All statistical analyses employed MINITAB (1986) software.

RESULTS

General Ecology

Salinity during the year in which the fish were collected ranged from 0 to 12 ppt. Water temperature ranged from 7 to 32°C. During the months of highest reproductive activity, April to June, salinity varied from 2 to 6 ppt and mean water temperature was 28°C. Day length increased from 12.48 hours on 1 April 1987 to 14.05 hours on 30 June 1987 (United States Naval Observatory 1965). In September, when there was a smaller peak in reproductive activity, the salinity was 12 ppt and water temperature was 28°C. Day length decreased from 12.81 hours on 1 September 1987 to 11.90 hours on 30 September 1987.

Specimens for preservation were collected in clam shells, algal mats, grass clumps, and *Phragmites australis* roots along the edge of the lake. Seining over soft sediments produced few specimens compared to the number collected close to the banks.

Life History

Length-frequency relationships examined by month indicated that *G. bosci* life expectancy was not more than 12 to 13 months (Figure 1). This conclusion was based on the fact that during the months of August and September specimens greater than 25 and 29 mm respectively were not collected. However, from November to June individuals exceeding 29 mm (range=30.0-44.4 mm) were common.

A broad range of size classes from December to April indicated that *G. bosci* most likely reproduced throughout the spring and summer. The smallest specimens (minimum=11.1 mm) were collected from August to October and represented offspring newly recruited into the population during late spring and summer. The maximum size of specimens progressively increased from October to April when a maximum size of 44.4 mm was reached. From April to June, the beginning of the reproductive season as discussed below, the size of *G. bosci* decreased from the maximum to 40.0 mm (Figure 1). The maximum size of *G. bosci* in August was 24.8 mm, in September, 29.7 mm, and in October, 36.6 mm. The increase in maximum size between each pair of consecutive months was 4.9 mm and 6.9 mm with an average of 5.9 mm/month. Thus if a growth rate of approximately 6 mm/month through the summer is assumed, the largest individuals in August would be no more than about four months old. These fish indicate that all gobies collected in this month represented a new generation.

Length-weight relationships comparing males and females showed that males not only reached a greater maximum standard length (44.4 mm) than females (34.7 mm), but were heavier per unit body length (Figure 2). A curvilinear relationship between standard length and weight existed for males and females. The regression analysis produced the following equations:

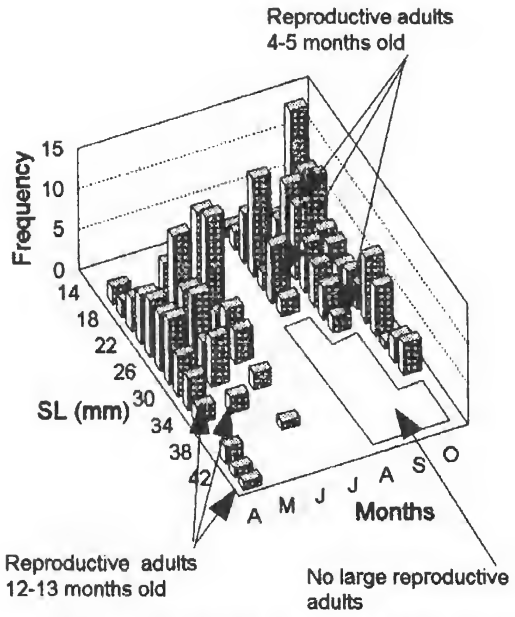

Figure 1. Length-frequency histogram for the months involved in the breeding season of *G. bosci* in the McFaddin National Wildlife Refuge, Sabine Pass, TX. Size class increments are based on 2 mm intervals. Data for the month of July are missing. The month of October is given for size comparisons, but is not part of the breeding season.

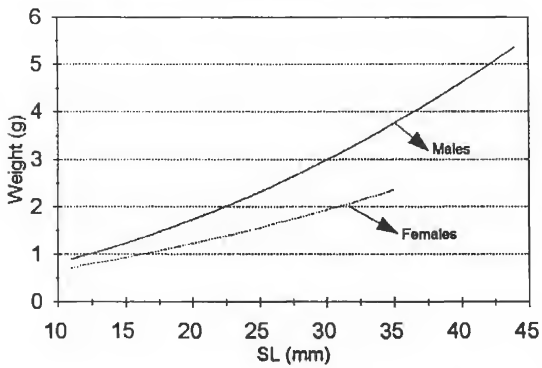

Figure 2. Length-weight relationships for male and female *G. bosci* are given. The 367 data points used to compute the regression lines are not shown.

$Weight_{males} = 0.281 + 0.0364SL + 0.0018SL2$

$Weight_{females} = 0.291 + 0.0309SL + 0.0008SL2$

These two regression models were significantly different (N=367; R^2=0.958; DF=3, 363; F=6.1891; P<0.005) and showed that males were heavier than females per unit length. The results of t-tests indicate that the quadratic (t=-4.03, P<0.01) and linear (t=3.27, P<0.01) components were responsible for the differences between the models.

Ova easily teased from the ovaries were classified as mature to ripe (Heins and Rabito 1986). Mean number of mature to ripe ova per female was 466 (N=40, SD=178.2) with a range of 116 to 1030. These ova made up two distinct size classes in each of six females examined from April and May (Figure 3).

Mean diameter for all stages of developing ova in May was 0.45 mm (N=12, SD=0.1153). The September mean ovum diameter was much lower, 0.19 mm, but the variance greater (N=8, SD=0.1538). Mean ovum diameter of mature and ripening eggs in April and May was 0.49 mm (range: 0.35 to 0.56 mm) and, in September, 0.42 mm (range: 0.34 to 0.50 mm). A two sample t-test showed a significant difference in the diameter of mature and ripening ova for the two periods (N=23; t=2.75; DF=21; P=0.012).

Not only were ovum diameters smaller during the second breeding peak, but ovum number per female was also significantly lower (N=29: DF=3, 25: R^2=0.73; F=22.42; P=0.0001). A regression analysis comparing egg number versus SL for females from April and May against females from August and September produced the following models:

$Eggs_{peak\ 1} = -577 + 45.4SL$

$Eggs_{peak\ 2} = -382 + 34.0SL$

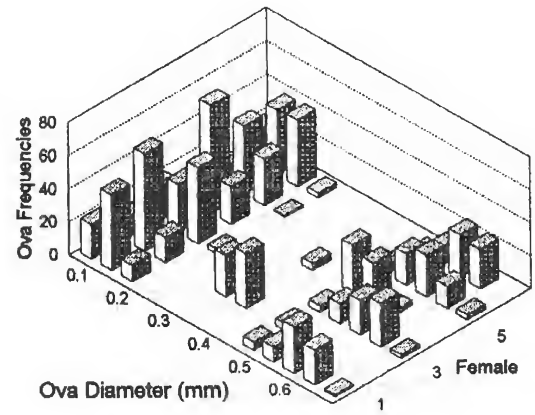

Figure 3. Ovum size class frequencies for six females collected in April and May. Size class increments are based on 0.05 mm intervals.

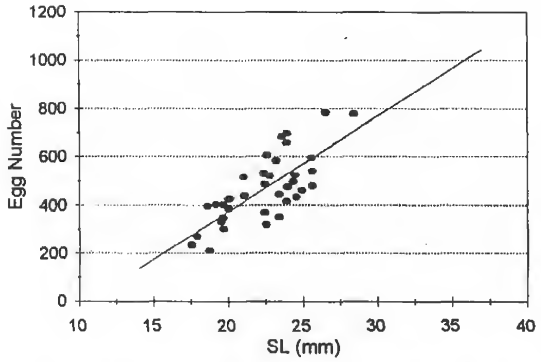

Figure 4. Linear relationship between SL and egg number for all females collected during the breeding season in the McFaddin National Wildlife Refuge, Sabine Pass, TX.

The difference between the two models resulted from significant differences in the coefficients of the slopes (t=2.22, P=0.036), but not in the intercepts (t=0.51, P=0.617). The mean egg number during the first peak was 505 (SD=137.1) and 252 (SD=95.2) during the second peak.

The total number of maturing to ripe ova in the ovaries of all females produced the following regression model (Figure 4):

Fecundity = -416 + 39.5SL

The positive correlation between SL and ova number was significant (N=37; DF=1, 35; F=45.836; P<0.001; R^2=0.567).

Mean male GSI values were low from November to January, began to rise in February and March, and peaked in April (Figure 5). GSI values then declined slightly during June, produced a second peak in August, and then declined in September. The April peak had a mean value of 1.266 and the August peak a mean of 1.284. Male GSI values peaked one month prior to the female peak GSI values for both reproductive periods.

Female GSI values showed a bimodal distribution, but with a more dramatic change than males (Figure 5). Female GSI values remained low November through December, began a gradual increase in January, increased rapidly February through April, and peaked in May at a mean value of 14.26. GSI values declined in August, peaked again in September at a mean value of 4.10 and declined in October.

In male *G. bosci* the AGS was mitten-shaped with a small lobe extending lateral to the main lobe, attached to the body wall near the vent, and extended dorsally between the inner body wall and the intestinal tract. Each AGS was attached to the caudal end of the testis by a small duct. During December the AGS were small and difficult to locate and remove. In months of high reproductive activity, the AGS were large and completely filled the ventral portion of the abdominal cavity.

AGSI values (Figure 5) followed a pattern similar to GSI values for males. AGSI values were low November through December and rose slowly from January to March. Unlike GSI values however, AGSI values did not peak in April but continued to climb and peaked in May, remained high through August, and then declined until October.

Ovarian maturation in female *G. bosci* was determined by ovum diameter. Mean ovum diameters (Figure 6) remained low October through February, and then began to increase in March. The increase in ovum diameter in March signaled the onset of ovum maturation. Therefore, the mean ovum diameter for March was used as a criterion against which ovum diameters for fish examined in other months were compared. All females whose mean ovum diameter was larger than 0.1924 mm, the mean ovum diameter for March, were considered to possess maturing ova.

Of 32 females examined in August and September, 13 had a mean ovum diameter greater than 0.1924 mm, the minimum mean size of maturing ova. The mean SL of these 13 females was 20.8 mm (range=16.9 to 24.0 mm). Of the 40 fish examined in April and May, 31 had ovum diameters exceeding 0.1924 mm. The mean standard length of these 31 females was 24.1 mm (range=18.9 mm to 29.6 mm). A comparison of SL indicated that sexually mature females from August and September were significantly shorter than females from April and May (N=22; DF=20; t=4.92; P=0.0001). In addition, significantly fewer females possessed maturing ovaries during the second breeding peak (N=32, DF=1, X^2=24.953, P<0.001).

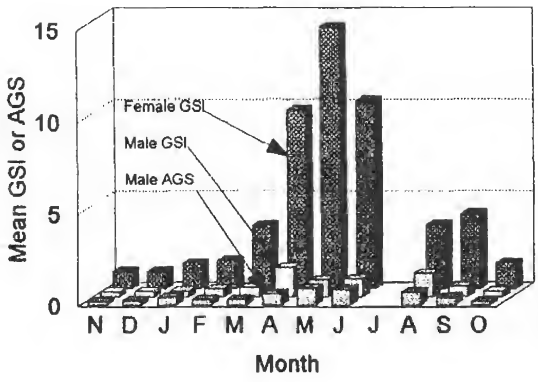

Figure 5. Monthly mean GSI and AGS values for males and females. Data for the month of July are not given.

Male sexual maturity was determined on the basis of GSI values (Figure 5). GSI values, which began increasing in March, were used as an indicator of maturing testes (N=16, $\bar{X}$=0.5624). Of 23 males examined in April, 20 had a GSI exceeding 0.5624. The mean SL of these 20 males was 30.6 mm (range: 19.5 to 44.4 mm). GSI also peaked in August. Mean SL of males with a GSI value above 0.5624 was 20.5 mm (range: 17.3 to 24.6 mm). A two sample t-test comparing maturing males from each time period indicated that males matured at a shorter SL in the late summer (N=61; DF=56.9; t=6.10; P=0.0001).

DISCUSSION

According to conclusions by Dahlberg and Conyers (1973), populations of *G. bosci* in the northern parts of its range should reproduce late in life after having grown and matured over the winter. Populations at the southern end of the range, such as in south Florida and Mexico, would be expected to mature earlier and possibly at a smaller size during the protracted breeding season. The primary factor controlling the age at first reproduction, and thus the reproductive tactic employed by an individual, would be the length of the breeding season in combination with the time of hatching.

The onset of the reproductive season in the Clam Lake population of *G. bosci*, determined by changes in GSI and AGSI values, and ovum diameters, indicated the following: (1) the reproductive season extended from at least late April to September, a period of five to six months; (2) the reproductive season possessed bimodal peaks in spawning activity; (3) the presence of mature and ripening ova from April to September indicated that breeding occurred throughout the summer; and (4) individual females possessed multiple size classes of ova suggesting that they might breed more than once during the season, a fact supported by field experiments (Conn 1989).

Individuals involved in the first reproductive peak in May were significantly larger than those in the September peak. The absence of large individuals in the last half of the summer indicated that the oldest individuals were not living more than 12 to 13 months and were dying out by August. Therefore, at least some individuals involved in the second breeding peak had hatched out during the early part of the breeding season, matured rapidly, and were breeding by the end of the breeding season. Among these individuals, only 40.6% produced eggs capable of being ovulated and laid, and indicating that the majority of individuals did not reproduce until the following spring.

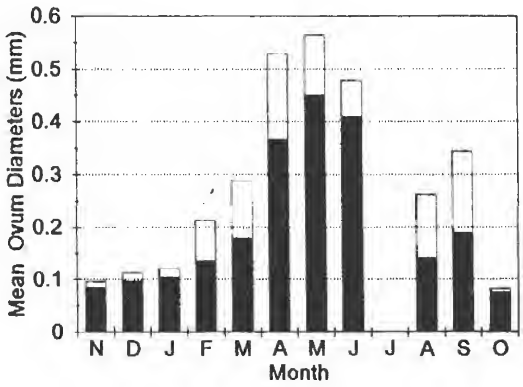

Figure 6. Monthly mean ovum diameters. Data for the month of July are not given. Open portions of bars represent standard deviations.

From this analysis we conclude that individual members of the *G. bosci* population in Clam Lake followed two distinct reproductive strategies based upon their time of hatching. Some individuals that hatched early in the breeding season matured rapidly, reproduced at the end of the breeding season, and laid a low number of eggs. These same individuals then had the opportunity for further growth over the winter with a second opportunity to produce a greater number of eggs in the early part of the next breeding season. Individuals hatched later in the reproductive season were too small to reproduce by the end of the season and delayed breeding until the following year. These conclusions support Stearns and Crandall (1984) and Stearns and Koella (1986), who found that plasticity in reproductive strategies allows individuals to vary their age and size at first reproduction. Our data also indicate that time of hatching is a critical factor in determining which reproductive strategy individual *G. bosci* employ in protracted breeding seasons.

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Cryopreservation of Sperm of Spotted Seatrout (*Cynoscion nebulosus*)

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CRYOPRESERVATION OF SPERM OF SPOTTED SEATROUT (*CYNOSCION NEBULOSUS*)

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ABSTRACT Cryopreservation of fish sperm has applications in preserving genetic resources from stocks of endangered fishes, replenishing fisheries, reducing the number of males needed in hatchery situations, and allowing repeated spawning of specific males. As part of a larger study on artificial breeding of sciaenid fishes, we developed procedures for collection, handling, refrigerated storage, and cryopreservation of spotted seatrout sperm. Hanks' balanced salt solution (HBSS) was used as an extender for collection and storage of sperm. Sperm motility in relation to graded concentrations of HBSS was used to determine the osmolality at which sperm were activated. Based on these findings, HBSS was prepared at 201 mOsm/kg as an extender for sperm storage. To determine if ions present in HBSS were involved in sperm activation, separate activating solutions were prepared by the addition of NaCl, $CaCl_2$, KCl, Na_2HPO_4, or $MgSO_4$ to aliquots of a stock glucose solution (185 mOsm/kg). The chemicals were added at the concentration of each found in 1-x HBSS. Only the glucose solution containing 8 g/l NaCl (424 mOsm/kg) produced activation of sperm. We also evaluated four chemicals as cryoprotectants: methanol, glycerol, dimethyl sulfoxide (DMSO), and n,n-dimethyl acetamide. Two freezing rates were evaluated by placing samples at either of two heights within a nitrogen vapor shipping dewar. The highest post-thaw motilities were in 10% DMSO with an average retention of 60% of initial motility at the lower position in the dewar, and 37% at the upper position. A third freezing rate was produced using a computer-controlled freezer programmed for a rate of -45°C/min, yielding a retention of initial motility of 31%. Our freezing and transport of cryopreserved sperm in shipping dewars demonstrate the utility of this procedure for field applications.

Introduction

The family Sciaenidae contains several species important to recreational and commercial fisheries. The red drum (*Sciaenops ocellatus*), black drum (*Pogonias cromis*), and spotted seatrout (*Cynoscion nebulosus*) all have large commercial fisheries that were closed or restricted to prevent overfishing. The decline of these fisheries has stimulated interest in development of methods such as artificial spawning and the use of cryopreserved sperm to aid in restoration efforts. Cryopreservation of sperm can be used to preserve genetic resources from stocks of fishes that are endangered and to aid in replenishing fisheries. Cryopreserved sperm can be used to reduce the number of males maintained in the hatchery and allows repeated spawning of specific males when females are in spawning condition. Cryopreservation can be used to preserve gametes of improved stocks, to study hybridization and crossbreeding, and to accelerate genetic research.

The first studies of cryopreservation of fish sperm were performed in marine fishes to aid in hybridization of herring stocks that spawned at different times of the year (Blaxter 1953). Most subsequent studies, however, have been of freshwater species, especially salmonids (see reviews by Scott and Baynes 1980; Stoss 1983). Previous work in reproductive biology of sciaenids has addressed natural spawning (Saucier and Baltz 1993), induced spawning (Colura 1974; Thomas and Boyd 1988), and hybridization (Henderson-Arzapalo and Colura 1984; Henderson-Arzapalo et al. 1994). Cryopreservation of sperm from the Atlantic croaker (*Micropogonias undulatus*) was studied by Gwo et al. (1991) and represents the only report on cryopreservation of sperm from a sciaenid species.

As part of a larger study on artificial breeding of sciaenids, we developed procedures for collection, handling, refrigerated storage, and cryopreservation of spotted seatrout sperm. Our objectives were to: 1) determine the relationship of osmotic pressure and sperm activation to allow safe storage; 2) evaluate the effect on sperm activation of specific ions used in the extender solution; 3) evaluate the effectiveness of different cryoprotectants; and 4) evaluate the success of different freezing rates. Motility estimates were used as a measure of sperm viability. To our knowledge, this is the first report of the cryopreservation of sperm of spotted seatrout.

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MATERIALS AND METHODS

Blood Plasma Osmolality and Extender Preparation

Blood samples were collected from 34 spotted seatrout caught from April-August, 1994 in Barataria Bay, LA (29°19' N, 89°56' W). Water in the Bay ranged in osmolality from 450-750 mOsm/kg during the collection period. The blood samples were allowed to clot, and 10 µl of plasma were used to determine osmolality with a vapor pressure osmometer (model 5500, Wescor Inc., Logan, UT). The osmolality of blood was 356.0 ± 18.4 mOsm/kg (mean ± SD). This value is similar to plasma values (350 mOsm/L) obtained for red drum (Crocker et al. 1983), another member of the family Sciaenidae. Sperm of marine species typically become motile in solutions of higher osmotic pressure than the blood plasma. Therefore, Hanks' balanced salt solution (HBSS) was prepared (Tiersch et al. 1994) using reagent grade chemicals (Sigma Chemical Corp., St. Louis, MO) at an osmotic pressure (300 mOsm/kg) below that of the blood plasma to ensure that sperm remained inactive when placed in the extender for storage.

Estimation of Sperm Motility

The percent motility of each sperm sample was estimated using darkfield microscopy at 100x immediately after addition of the activating solution. Percent motility was defined as the percentage of progressively motile sperm within each activated sample. The osmolality of the activated sperm mixture was determined by removing 10 µl of diluted sample directly from the microscope slide for analysis by a vapor pressure osmometer. The threshold activation point was defined as the osmotic pressure at which 10% of the sperm became motile. The complete activation point was defined as the lowest osmotic pressure that elicited the highest percentage of motile sperm.

Osmotic Analysis of Sperm Activation

Sperm samples were collected by manual stripping of three males caught in April 1994 in Barataria Bay, Louisiana. For this, fish were dried with a towel, and gentle pressure was applied to the abdomen. Sperm were collected in 75-µl hematocrit tubes, transferred to 1.8-ml centrifuge tubes, and diluted with 1 ml of HBSS (300 mOsm/kg). Sperm activation was evaluated according to Bates et al. (1996) by dilution of 2-µl aliquots of sperm with 20 µl of solutions ranging in osmotic pressure from deionized water (8 mOsm/kg) to double-strength HBSS prepared at 600 mOsm/kg. Because HBSS is highly ionic, solutions of mannitol (Sigma) were prepared at three concentrations (200, 300, and 350 mOsm/kg) and used to test activation of sperm in solutions deficient in ions.

Ionic Analysis of Sperm Activation

Because there was a persistent low level of spontaneous motility (1%) of sperm placed in the HBSS extender solutions, we tested storage of sperm in HBSS at osmotic pressures as low as 152 mOsm/kg. To determine if ions present in HBSS were involved in sperm activation, separate activating solutions were prepared by addition of NaCl, $CaCl_2$, KCl, Na_2HPO_4, or $MgSO_4$ to aliquots of a stock glucose solution (185 mOsm/kg). The chemicals were added at the concentration of each found in 1-x HBSS. Glucose and sucrose solutions prepared at higher osmolalities were used as control treatments to test effects on sperm activation (Table 1), and motility estimates were performed as described above. Artificial seawater (Forty Fathoms Bio-crystals Marinemix, Marine Enterprises International, Inc., Baltimore, MD) prepared at an osmolality of 628 mOsm/kg was used to establish the level of complete motility.

Evaluation of Cryoprotectant Toxicity

Initial cryopreservation studies were performed in the field at the Louisiana Department of Wildlife and Fisheries Lyle S. St. Amant Marine Biological Laboratory on Grand Terre Island. Sperm from two males caught on April 9 were collected by surgical removal and smashing of testis. The sperm were stored in HBSS (186 mOsm/kg) at 4°C. We evaluated four reagent-grade chemicals (Sigma) as cryoprotectants: methanol, glycerol, dimethyl sulfoxide (DMSO), and n,n-dimethyl acetamide (DMA). Each cryoprotectant was mixed at 50%:50% (v:v) with HBSS before addition to sperm mixtures. All cryoprotectants were used at concentrations of 5% and 10% except DMA, which was only used at a 5% concentration because of acute toxicity at higher concentrations (data not shown). The time between addition of cryoprotectant to the sperm and initiation of the freezing procedure was 15 min. Motility was estimated at the initiation of the freezing procedure to determine the acute toxicity of each cryoprotectant to spotted seatrout sperm. The sperm used in this study were subsamples of the samples used in the cryoprotectant toxicity and freezing rate study described below.

Evaluation of Cryoprotection and Freezing Rates

Sperm were cryopreserved in 0.5-ml straws (IMV International Corp., Minneapolis, MN) with two replicates per fish (n = 2) for each treatment. Straws were sealed using glass balls (Minitube of America, Madison, WI), and were placed into an RPE embryo freezer (Peter Elsden and Associates, Collierville, TN) designed for the cryopreservation of mammalian embryos. The RPE embryo freezer consisted of a metal cylinder, with holes drilled for sixteen 0.5-ml straws, designed to create a uniform freezing

rate when lowered into nitrogen vapor in a vapor shipping dewar (Model CP-35, Taylor-Wharton, Theodore, AL). Two freezing rates were accomplished by placing the freezer at either of two heights within the dewar. Placing the center of the RPE embryo freezer 220 mm from the bottom of the dewar yielded the fastest freezing rate; placement 320 mm from the bottom of the dewar yielded a slower freezing rate. To document the freezing rates, a straw filled with HBSS was inserted into the freezer and the temperature was recorded using a type-T thermocouple and a strip chart recorder. The recording was initiated at the time the straws were placed into the RPE embryo freezer and ended when the straws were removed (30 min after reaching -80°C). Straws were transferred immediately to a larger shipping dewar (Model CP-65, Taylor-Wharton) for storage. After 72 hours of storage, the straws were thawed for 7 sec in a water bath at 40°C. The straws were dried and the ends cut to release the sperm into 1.8-ml tubes. For estimation of sperm motility, a 2-µl aliquot of each sample was activated with 20 µl of HBSS (600 mOsm/kg) to obtain maximal motility.

In an experiment performed in the laboratory at Louisiana State University, a computer-controlled freezer (Kryo-10, Planer Products Ltd., England) was used to produce a third freezing rate (-45°C/min). Sperm from the two males used for the dewar studies were transported at 4°C and stored for 24 hr before analysis. Dimethyl sulfoxide at 10% was chosen as the cryoprotectant for this experiment, based on results of the experiments performed in the shipping dewar. Straws were frozen using a two-step procedure. The straws were first cooled to a temperature of 5°C for 5 min, and then frozen at a rate of -45°C/min until reaching -80°C. The straws were maintained at -80°C for 20 min, removed from the freezer, and plunged immediately into liquid nitrogen for storage. After 48 hr of storage, sperm samples were thawed as described above and motility estimated.

TABLE 1

Activation of spotted seatrout sperm. Sperm were stored at 4°C in Hanks' balanced salt solution (HBSS) at either 152 mOsm/kg or 201 mOsm/kg for 2 days prior to analysis. Two-µl aliquots of sperm were activated with 20 µl of activating solution. Motility was estimated under 100x dark-field microscopy. Sugar solutions and artificial seawater were used to establish control values. Solutions containing ionic components of HBSS were prepared in a glucose solution (185 mOsm/kg) to supplement osmotic pressure.

Storage solution	n	Activating solution ingredients(osmolality)	Final osmolality[1]	Percent motility
152	2	Artificial sea water (628 mOsm/kg)	585	18
201	2		597	35
152	2	Sucrose (757 mOsm/kg)	724	18
201	2		713	35
152	1	Glucose (321 mOsm/kg)	318	5
152	1	Glucose (258 mOsm/kg)	258	1
152	2	Glucose (207 mOsm/kg)	211	0
201	2		214	0
152	1	Glucose + 8g/L NaCl	424	10
201	1	Glucose + 0.16g/L CaCl	189	0
201	1	Glucose + 0.4 g/L KCl	199	0
201	1	Glucose + 0.06 g/L Na_2HPO_4	183	0

[1] mOsm/ Kg

Statistical Analysis

All percent motility values were arcsine-squareroot transformed prior to statistical analysis. Motility data derived from the osmotic analysis of sperm activation were compared using a paired Student's t-test (Microsoft Excel 5.0, Microsoft Corp.). In the cryoprotectant toxicity study, differences in pre-freezing motility were determined using a one-factor analysis of variance (SAS 6.08, SAS Institute Inc., Cary, NC). In the study evaluating post-thaw motility as a function of cryoprotectant and freezing rate, differences were determined using a two-factor analysis of variance (SAS 6.08). Means were separated using Duncan's multiple range test, and were considered significant when $P \leq 0.05$.

RESULTS

Osmotic Analysis of Sperm Activation

The motility of spotted seatrout sperm increased with increased osmolality of the HBSS or mannitol activating solutions, with maximum motility (90%) observed at ~375 mOsm/kg and above (Figure 1). Motility of sperm activated in mannitol solutions was not significantly different ($P > 0.27$) from motility of sperm activated in HBSS. In general, ~5% of the sperm (subthreshold) became motile at 242 mOsm/kg. The threshold activation point (10% motility) was 262 mOsm/kg, and the complete activation point (90% motility) was 370 mOsm/kg.

Ionic Analysis of Sperm Activation

To evaluate the activating effect of the various ions contained in HBSS, individual chemical components of HBSS (at the concentration used in 1-x HBSS) were dissolved in glucose solutions (prepared at 185 mOsm/kg to supplement the osmotic pressure) and used as activating solutions for spotted seatrout sperm. Only the glucose solution containing 8 g/l NaCl produced sperm activation. The osmolality of this solution was 424 mOsm/kg (Table 1), above the complete activation point identified in Figure 1. Sperm activated by sucrose solutions at osmolalities of 724 mOsm/kg produced motility equal to that of sperm activated by artificial seawater at 628 mOsm/kg (Table 1). Spotted seatrout sperm stored at 152 mOsm/kg demonstrated reduced motility of as much as 50% compared to sperm stored at 201 mOsm/kg.

Evaluation of Cryoprotectant Toxicity

The average initial motility of sperm samples was 75% at the time of addition of cryoprotectants. Sperm motility at the time of freezing was reduced significantly ($P = 0.0001$) by exposure to glycerol and DMA (Table 2). This loss of motility was likely due to acute toxic effects of the chemicals on sperm. Of the four cryoprotectants, exposure to glycerol reduced pre-freeze motility the most (to ~1%). Pre-freeze motility of sperm exposed to methanol or DMSO was not significantly different ($P > 0.05$) from motility of control sperm not exposed to cryoprotectants.

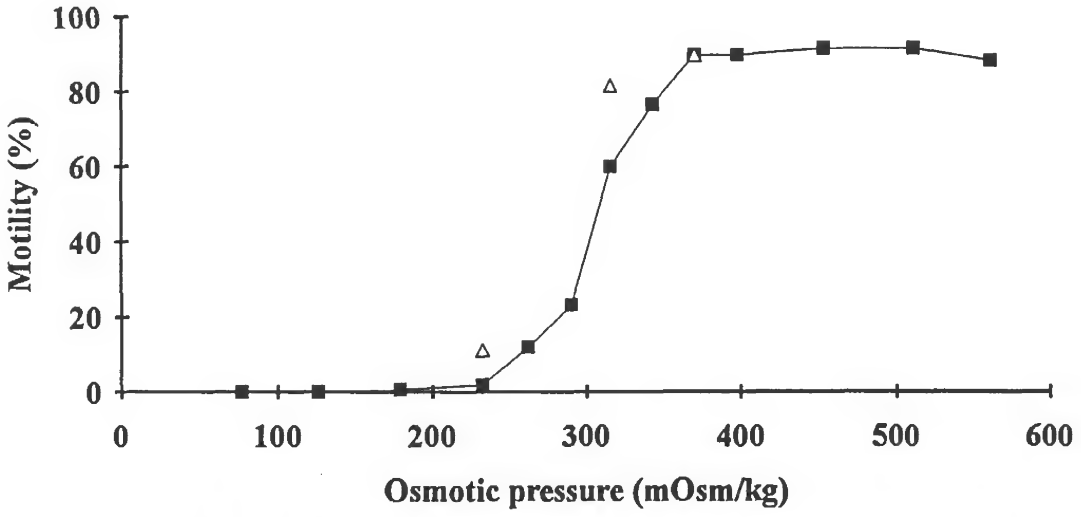

Figure 1. Percent of motility of spotted seatrout sperm activated with solutions of Hanks' balanced salt solution (squares) or mannitol (triangles) spanning a range of osmotic pressures. Each point represents the mean of sperm from three fish. Motility of sperm activated with mannitol was not significantly different ($P > 0.05$) from motility of sperm activated with HBSS at corresponding osmotic pressures. All standard errors were less than 10%.

TABLE 2

Mean motility[1] (± SD) before freezing and after thawing of sperm of spotted seatrout (n=2). Sperm was frozen at one of two positions in a nitrogen vapor shipping dewar: lower (-3.5 °C/min) and upper (-2.5 °C/min); or in a computer-controlled freezer (-45.0 °C/min). Sperm frozen in 10% DMSO had significantly higher (P=0.001) post-thaw motility than sperm frozen in other cryoprotectants. Position within the dewar had no significant effect on post-thaw motility (P=0.15). Pre-freeze motility values sharing a letter were not significantly different.

Cryoprotectant	Concentration	Pre-freeze motility[2](%)	Post-thaw motility (%)		
			Lower	Upper	Freezer
Control[3]	--	70 ± 0[a]	0 ± 0	0 ± 0	--
Methanol	5%	75 ± 6[a]	1 ± 1	0 ± 0	--
	10%	75 ± 6[a]	0 ± 0	0 ± 0	--
DMSO	5%	65 ± 6[ab]	13 ± 4	13 ± 11	--
	10%	63 ± 3[ab]	45 ± 21	28 ± 11	--
	10%	72 ± 4	--	--	22 ± 18
DMA	5%	49 ± 25[b]	3 ± 3	1 ± 0	--
Glycerol	5%	1 ± 1[c]	1 ± 0	1 ± 0	--
	10%	1 ± 1[c]	1 ± 0	1 ± 0	--

[1] Initial motility at time of collection was >75%.
[2] Motility estimated 15 min after the addition of cryoprotectant.
[3] Hanks' balanced salt solution without cryoprotectant.

Evaluation of Cryoprotection and Freezing Rate

Ten percent DMSO produced the highest post-thaw motility of the four cryoprotectants studied (P = 0.001). Sperm frozen in other cryoprotectants yielded motilities of between 0% and 3%. Samples frozen without any cryoprotectant contained no motile sperm after thawing.

The average rates of freezing for the shipping dewar were -3.5°C/min for the lower position and -2.5°C/min for the upper position. The highest post-thaw motilities were in 10% DMSO with an average retention of 60% of initial motility when frozen at the faster rate, and 37% at the slower rate (Table 2). Freezing rate in the shipping dewar had no significant effect on the post-thaw motility (P = 0.15). Sperm frozen in the computer-controlled freezer(-45°C/min) retained an average of 31% of initial motility.

DISCUSSION

The osmolality of blood plasma (~350 mOsm/kg) was used as an estimator of the osmolality of seminal plasma to allow development of preliminary extender solutions for sperm storage. Our preliminary extender allowed storage for 24 h, but did not completely inhibit sperm activation. In a study on the cryopreservation of Atlantic croaker sperm, Gwo et al. (1991) used various extenders to freeze sperm. Although motility in the extenders was not mentioned, 1% unbuffered NaCl yielded the highest post-thaw fertilization rate. A study on cryopreservation of gilthead seabream (*Sparus aurata*) (Chambeyron and Zohar 1990) used two extenders developed by Billard (1984) in previous work with gilthead seabream. In these extenders, sperm motility decreased as the osmolarity of the extender neared the osmolarity of the blood (364 mOsm/l), but motility was observed in all samples after 1-2 min at osmolarities as low as 303 mOsm/l.

In the present study, an activation curve was used to determine the osmolality at which sperm became motile in HBSS. In general, sperm increased in motility as the activating solution increased in osmotic pressure. Other studies on marine fish sperm show the same relationship between sperm activation and increased osmotic pressure. For example, sperm from the pikey bream (*Acanthopagrus berda*) showed no activation in 0 ‰ to 5 ‰ seawater and highest motility in 35 ‰ seawater (Palmer et al. 1994). We used mannitol solutions to determine if a change in ionic concentration was involved in sperm activation. The curve generated for mannitol was not significantly different from that for HBSS (over the tested range of 232-370 mOsm/kg). These studies prompted us to reformulate the extender solution at an osmolality (200 mOsm/kg) that was below the threshold activation point. This extender did not completely prevent activation of sperm, but did reduce the level of spontaneous motility to ~1% and allowed storage of sperm for 3 d at 4°C. Sperm storage in solutions of HBSS with osmolality as low as 152 mOsm/kg did not prevent spontaneous activation and reduced sperm motility after storage at 4°C by as much as 50%, indicating that storage in buffers of low osmotic pressure may be detrimental to sperm.

Activation experiments were also performed with solutions of sucrose, glucose, and glucose supplemented with individual components of HBSS. Activation of sperm in glucose supplemented with ionic components of HBSS was not observed at osmotic pressures below 200 mOsm/kg, suggesting that the ions in 1-x HBSS were not the cause of the spontaneous activation observed during storage. Activation of spotted seatrout sperm may be triggered by changes in concentration of particular ions, osmotic pressure, or a combination of these factors. Further elucidation of the factors involved in the activation of spotted seatrout sperm would be useful for improvement of extender solutions.

Sperm from spotted seatrout were frozen in four cryoprotectants at two concentrations and at two freezing rates. The cryoprotectant yielding highest post-thaw motility was DMSO at 10% when a freezing rate of -3.5°C/min was used, although faster rates of freezing have been shown to be successful in the cryopreservation of Atlantic croaker sperm (Gwo et al. 1991). Sperm frozen in the computer-controlled freezer at -45°C/min retained a lower post-thaw motility than sperm frozen at either dewar freezing rate. Perhaps a freezing rate between -3.5°C/min and -45°C/min could increase the post-thaw motility of spotted seatrout sperm. A faster freezing rate in the shipping dewar could be obtained by freezing the straws in a suspended canister without the use of the RPE embryo freezer. Our freezing and transport of cryopreserved sperm in shipping dewars demonstrates the utility of this procedure for application to work outside of the laboratory.

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Aricidea (Allia) bryani, a New Species of Polychaete (Polychaeta: Paraonidae) from the Northern Gulf of Mexico

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ARICIDEA (ALLIA) BRYANI, A NEW SPECIES OF POLYCHAETE (POLYCHAETA: PARAONIDAE) FROM THE NORTHERN GULF OF MEXICO

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ABSTRACT *Aricidea bryani*, a new species of polychaete (Polychaeta: Paraonidae) belonging to the subgenus *Allia* Strelzov 1973, is described from shallow subtidal sediments along the northern shore of Mississippi Sound, an estuary of the northern Gulf of Mexico. The new species is distinguished from other members of the subgenus by the presence of tuberculate neuropodial lobes in the anterior 15-20 setigers, a cirriform median antenna that extends posteriorly to setiger three, and by modified neuropodial setae that are abruptly tapered at mid-length, but lack terminal aristae.

Introduction

Specimens of an undescribed species of *Aricidea* Webster 1879 (subgenus *Allia* Strelzov 1973) were collected near the historic Biloxi Lighthouse (Harrison County, Mississippi) during January 1991 to April 1992. The collection site (type locality) was a shallow, subtidal, mesohaline habitat in water less than 2 m deep. Collections were made adjacent to a fishing pier that extended seaward from an artificial, public beach on the northern shore of Mississippi Sound, an estuary of the northern Gulf of Mexico.

Aricidea Webster, 1879, is one of six genera in the family Paraonidae Cerruti, 1909; the others are *Paraonis*, *Paraonides*, *Cirrophorous*, *Levinsenia*, and *Sabidius*. Based on the presence and characteristics of modified neurosetae, Strelzov (1973) proposed subdividing the genus *Aricidea* into four subgenera as follows: *Aricidea* s. str; *Aedicira* Hartman, 1957; and two new subgenera, *Allia* and *Acesta*. Fauchald (1977) elevated Strelzov's four subgenera to generic rank without giving specific reasons, and Hartley (1981) corrected the subgenus name of *Acesta* to *Acmira*, since the former was preoccupied in the phylum Mollusca. The taxonomic scheme used here follows Strelzov (1973) and Hartley (1981).

Holotypes, paratypes, and additional material from the type locality are deposited in the U.S. National Museum of Natural History, Smithsonian Institution (USNM), Washington, DC. Other specimens are deposited in the museum of the Gulf Coast Research Laboratory (GCRL), Ocean Springs, Mississippi, and in the personal collections of the authors.

Taxonomy

Family Paraonidae Cerruti, 1909
Genus *Aricidea* Webster, 1879
Subgenus *Allia* Strelzov, 1973
***Aricidea (Allia) bryani*, new species**
Figures 1-4

Synonym: *Aricidea* cf. *alisdairi* (Gaston 1984, in part).

Material Examined

Type material. Northern Gulf of Mexico, Biloxi, Mississippi (30°10'N, 88°53'50"W). Holotype 22 mm length, 1.0 mm width (USNM 172124), 4 paratypes (USNM 172125), June 30, 1991; 26 paratypes from two collections made on March 28, 1991 (USNM 172126) and April 2, 1992 (USNM 172127), shallow subtidal (0.5-1.0 m), sediment type: well-sorted fine sand; all type material collected by K. Matulewski.

Additional material. Apalachee Bay, FL (30°1.5'N, 84°16.2'W): 5 specimens collected during July 1991, 4.6 m depth, muddy sand (83% sand); Oyster Bay, FL (30°3.0'N, 84°17.2'W): 4 specimens collected during July 1991, 2.1 m depth, sandy mud (57% silt-clay); Mississippi Sound, MS (30°15.3'N, 88°26.1'W): 1 specimen collected during July 1991, 4.9 m depth, sandy mud (90% silt-clay); Chandeleur Sound, LA (29°58.2'N, 88°51.1'W): 5 specimens collected during July 1991, 2.4 m depth, muddy sediments (96% silt-clay); Terrebone Bay, LA (29°45.0'N, 90°15.0'W): 5 specimens collected during July 1991, 2.3 m depth, muddy sediments (95% silt-clay); Caracahua Bay, TX

(28°37.6'N, 96°22.5'W): 1 specimen collected during July 1991, 1.2 m depth, sandy mud (73% silt-clay); Off St. Petersburg, FL (27°57'00.4"N, 83°09'00.3"W) (USNM 090223): 1 specimen collected during July 1976, 19 m depth, fine-very fine sand; Off St. Petersburg, FL (27°56'00.5"N, 83°27'29.6"W) (USNM 090216): 1 specimen collected during August 1977, 30 m depth, clayey, sandy silt; Off St. Petersburg, FL (27°52'30.5"N, 83°33'59.0"W) (USNM 090217): 1 specimen collected during August 1977, 34 m depth, clayey, sandy silt; Off St. Petersburg, FL (27°57'28.8"N, 83°42'29.2"W) (USNM 090218): 1 specimen collected during July 1976, 37 m depth, silty-very fine sand; Off Apalachicola, FL (29°30'N, 84°27'W) (USNM 090219): 1 specimen collected during July 1976, 24 m depth, medium-fine sand.

DESCRIPTION

Based on holotype and paratype material. Body dorsoventrally flattened in branchial region, more cylindrical in postbranchial region. Prostomium triangular, bluntly pointed anteriorly (Figure 1A). Nuchal slits very slender, inconspicuous, directed anterolaterally. Median antenna cirriform, widest near base, extending to anterior edge of setiger 3 (Figure 1B). Two small eyes present, faint in preserved specimens. Branchiae beginning on setiger 4, numbering 25-33 pairs on large specimens, 14-18 on very small specimens. Branchiae broad basally, tapering to slender, bluntly pointed terminus; overlapping across dorsum and perpendicular to body axis (Figures 1, 2). Branchiae subequal, except those of last pair, which are similar in shape and smaller, about 2/3 length of others (Figures 2D, 4A). Ciliated bands occurring across dorsum between pairs of branchiae and on branchiae, but less developed in the last pair (Figure 4A). Notopodial postsetal lobes digitate on setigers 1 and 2 (slightly longer on setiger 2), twice as long on setiger 3 and thereafter, thinner in postbranchial region to pygidium (Figures 2, 3). Neuropodial postsetal lobes tuberculate from setiger 1 to about setiger 18, absent on setiger 20 and thereafter (Figures 1, 2, 3). Notosetae all thin and capillary, numbering about 22-25 per fascicle in prebranchial and branchial region and about 8-13 per fascicle in postbranchial region. Neurosetae number about 22-28 per fascicle in prebranchial and branchial region and about 35 per fascicle in postbranchial region, stouter and somewhat shorter than notosetae in prebranchial region and anterior two-thirds of branchial region (Figures 1, 2); becoming thin, similar to notosetae in posterior third of branchial region and posteriorly to about setiger 60 at which point fascicles include setae abruptly tapering at about midlength with pubescent fringe on setal shaft where abrupt tapering occurs (Figure 3C); pubescent fringe extending from midlength to terminus of shaft, forming sharp tip (Figure 4B); abruptly tapering neurosetae becoming more numerous in far-posterior setigers (Figure 3B), comprising most of setae in fascicles near the pygidium. Body narrowing at pygidium to half its postbranchial width. Pygidium with two ventrolateral and one ventromedial anal cirri; ventromedial cirrus about half the length of two lateral cirri (Figure 1C).

Variation. The median antenna generally is cirriform, but antennae of some paratypes have a greater swelling near the base than that of the holotype. Additionally, some small (young) specimens have short, tuberculate antennae that appear to be in a developmental stage. Specimens from offshore Florida have similar numbers of branchiae, similar notopodial and neuropodial lobes, similar neurosetae and notosetae, but shorter antennae than on other specimens of *A. bryani* (to setiger 1 in USNM 090216, 090217, 090218, 090223; to middle of setiger 2 in USNM 090219).

Remarks. *Aricidea bryani*, n. sp., belongs to the subgenus *Allia*, which currently has 17 species characterized by the presence of neurosetae that are markedly thicker than corresponding notosetae. These characteristic neurosetae have abruptly tapering shafts, with more abrupt tapering in far posterior segments. *Aricidea (Aricidea) fragilis*, which is the type species for the genus, has similar neurosetae to species of *Aricidea (Allia)*, but is distinguished by the presence of pseudocompound (pseudoarticulate) neurosetae in some posterior segments (see Hartman 1957: 317). Although we were not able to examine type specimens, our examination of *A. fragilis* specimens from offshore North Carolina (USNM 51181), from Pivers Island near Beaufort, North Carolina, and from Perdido Key, Florida, revealed that all had the characteristic pseudocompound neurosetae in posterior segments. We are inclined, therefore, to maintain the subgenus *Allia* based on its modified setal type as set forth by Strelzov (1973). *Aricidea (Aricidea) fragilis* is otherwise distinguished from *A. bryani* by the presence of short (not tuberculate) neuropodial lobes to setiger 40 (not to 20), and by its short, subulate median antenna, which extends at most to setiger 2 (Webster 1879, Pettibone 1965:129).

Comparative characteristics of the species of *Aricidea (Allia)* are summarized in Table 1. Four species of *Allia*, like *A. bryani*, have neuropodial lobes and a cirriform antenna that extends posteriorly to at least setiger 3, but differ from the new species in the following respects: *A. abranchiata* lacks branchiae; *A. quadrilobata* has a long, filamentous antenna extending to setiger 9, well-formed, digitate neuropodial lobes anteriorly, and biramous notopodial cirri in the branchial region of larger specimens; *A. suecica* (= *A. nolani*; Strelzov 1973) has modified setae with terminal aristae on posterior segments; *A. pseudanne*

TABLE 1

Species of *Aricidea*, subgenus *Allia* with some comparative morphological characteristics for specimens of maximum size. Includes data from Hasan (1960), Pettibone (1963), Hartman (1965), Day (1967), Imajima (1973), Strelzov (1973), Katzmann and Laubier (1975), Hartley (1984), and Gaston (1984).

Species	Neuropodial lobes		Antenna		Pairs of branchiae
	type	end at setiger	type	end at setiger	
A. marianne Katzman & Laubier, 1975	tuberculate	13	short, ovoid, digitate tip	0	12-19
A. bulbosa Hartley, 1984	tuberculate	15	short, fusiform	0	21
A. albatrossae Pettibone, 1957	tuberculate	12-25	short, subulate	0	26-30
A. hartmani (Strelzov, 1968)	tuberculate	12	short, conical-clavate w/cerratophore	0-2	15-19
A. ramosa Annenkova, 1934	tuberculate	17	short, branched	1*	13-17
A. roberti Hartley, 1984	tuberculate	17	short, cylindrical	1	22-26
A. claudiae Laubier, 1967	tuberculate	9	basally enlarged, sharply attenuated tip	1	16
A. curviseta Day, 1963	tuberculate	20	cirriform	1	44
A. bryani n. sp.	tuberculate	18-20	cirriform	3	25-33
A. abranchiata Hartman, 1965	tuberculate	2-4	cirriform	4-7	0
A. suecica Eliason, 1920	tuberculate	25	cirriform	5	30
A. pseudanne Katzmann & Laubier, 1975	tuberculate	5-6	cirriform	5-6*	13
A. quadrilobata Webster & Benedict, 1887	conical, fusiform	25	cirriform, slender	9	27
A. monicae Laubier, 1967	absent	–	cirriform	0	9
A. facilis Strelzov, 1973	absent	–	short, club-shaped	0	9-15
A. pulchra Strelzov, 1973	absent	–	cirriform	1	18
A. alisdairi Hasan, 1960	absent	–	cirriform	2	43
A. trilobata Imajima, 1973	absent	–	long, clavate	2	20

* Based on illustration

is smaller, has an antenna extending to setigers 5-6, only 6-13 pairs of branchiae, and has neuropodial lobes only on the first six setigers. Two other species are similar to *A. bryani*, but are distinguished as follows: *A. roberti* has a short, cylindrical antenna, fusiform notopodial postsetal lobes on first two setigers (longer than those of *A. bryani*), and neuropodial lobes to setiger 17; *A. bulbosa* has bulbous posterior branchiae.

The new species was originally described as *Aricidea* (*Allia*) cf. *alisdari* by Gaston (1984), but not all of Gaston's specimens were determined to be *A. bryani*. Some specimens had similar notosetae and neurosetae to *A. bryani*, but longer notopodial lobes in the first two setigers and either shorter antennae (USNM 090220, 090222) or longer antenna (USNM 090221) than *A. bryani*. Another specimen (USNM 090215) had a short, clavate antenna (to setiger 1) and pseudoarticulate neurosetae in the posterior region.

Ecology. *Aricidea (Allia) bryani* was abundant in the littoral zone of Mississippi Sound. Specimens at the type locality occurred in densities of 28-555/m^2 during January 1991 to April 1992 (Matulewski 1995). Sediments of the area consisted primarily of well-sorted fine sand that periodically contained considerable amounts of silt and detritus. Most preserved specimens of the new species had a distinct corkscrew shape, and juveniles almost always were green, possibly due to ingested algae. Adults were whitish with an orange-brown stripe dorsally in some specimens. Presence of small specimens in the monthly collections at the type locality led the authors to conclude that larval settlement occurred from January to March during 1991. Only large specimens (adults) were collected during the summer. Specimens from the type locality were usually filled with ingested sand grains that were packed in the far-posterior gut. A combination of the filled gut and the

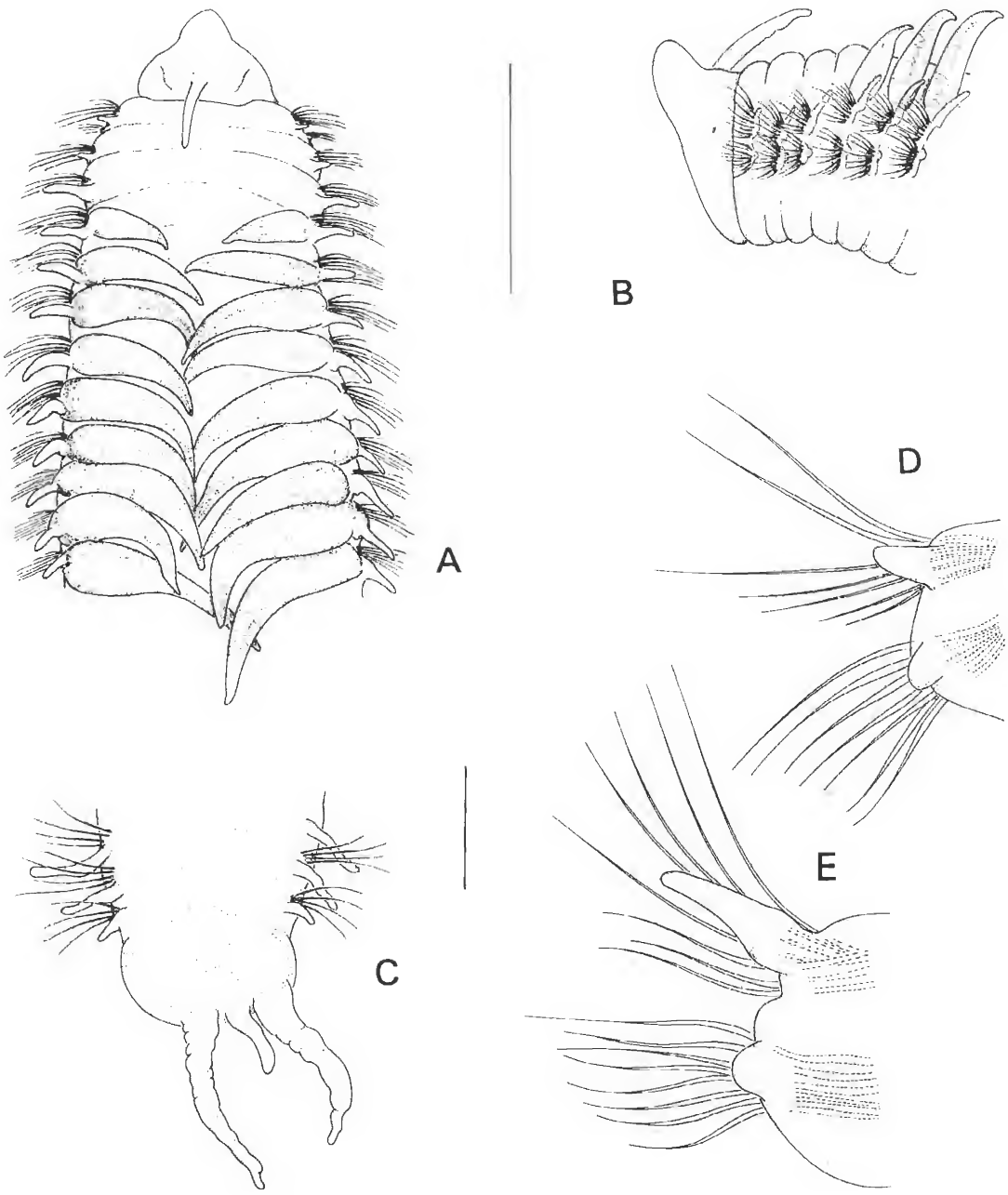

Figure 1. ***Aricidea (Allia) bryani*****, new species. A. Anterior end, dorsal view. B. Anterior end, lateral view. C. Pygidium (holotype), dorsal view. D. Setiger 1. E. Setiger 3. Parapodium in posterior view, number of setae reduced for clarity. Scales: A, B = 0.5mm; C, D, E = 0.1mm.**

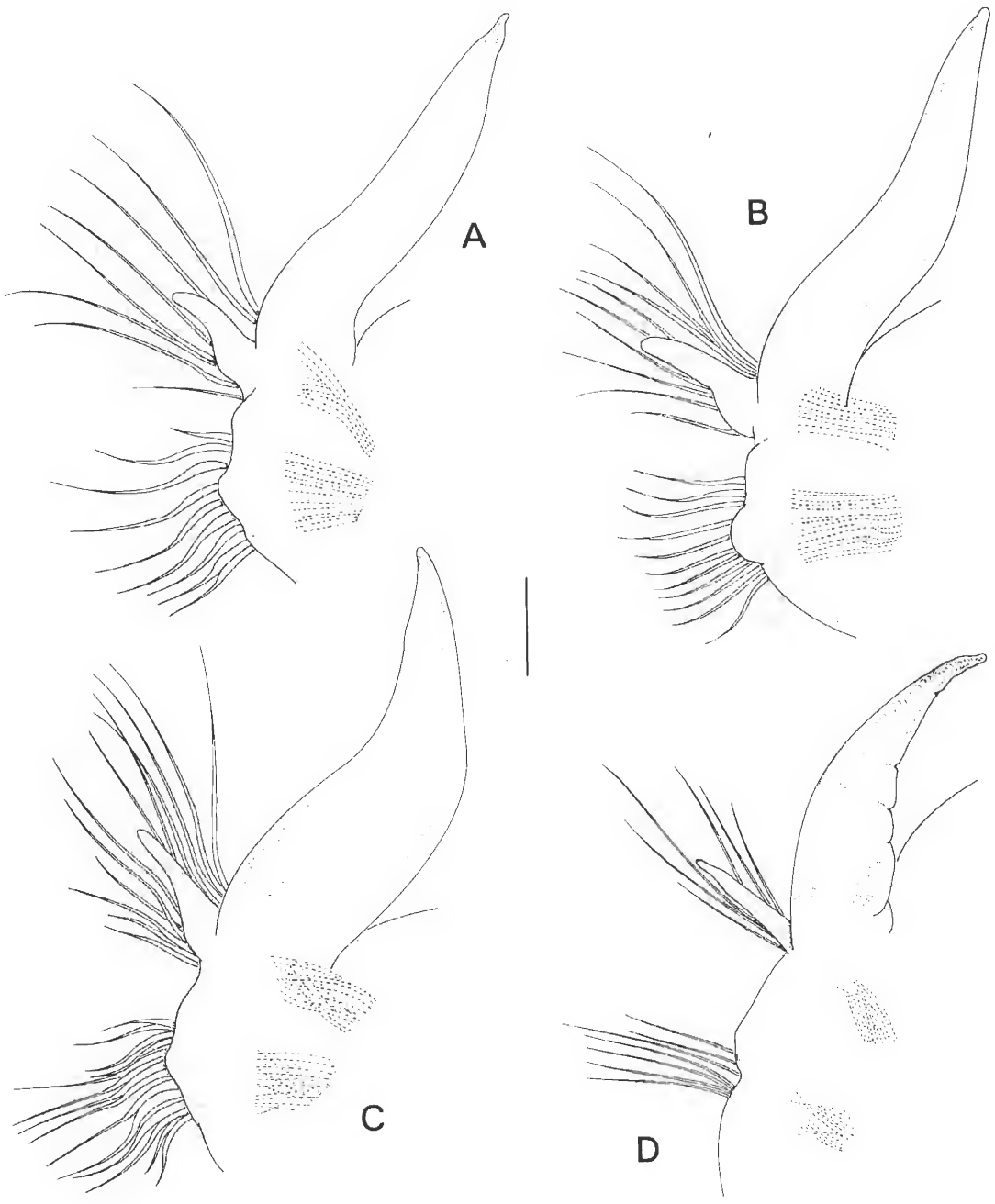

Figure 2. ***Aricidea (Allia) bryani*****, new species. Representative branchial parapodia, posterior views, number of setae reduced for clarity. A. Setiger 6. B. Setiger 9. C. Setiger 16. D. Setiger 29. Scale = 0.1mm.**

thin body wall resulted in loss of the far-posterior region and pygidium of most specimens during sample preparation. *Aricidea (Allia) bryani* was one of over 40 species of polychaetes that inhabited the type locality.

Ciliated bands across the dorsum and on the branchiae (Figure 4A) probably function in motility, enhance oxygen uptake, and may help keep the body surface free of debris. Similar bands were evident in scanning electron micrographs of *Aricidea roberti* and *Aricidea suecica* (Hartley 1984).

Etymology. The species is named in honor of Bryan Deaver Gaston, son of the senior author.

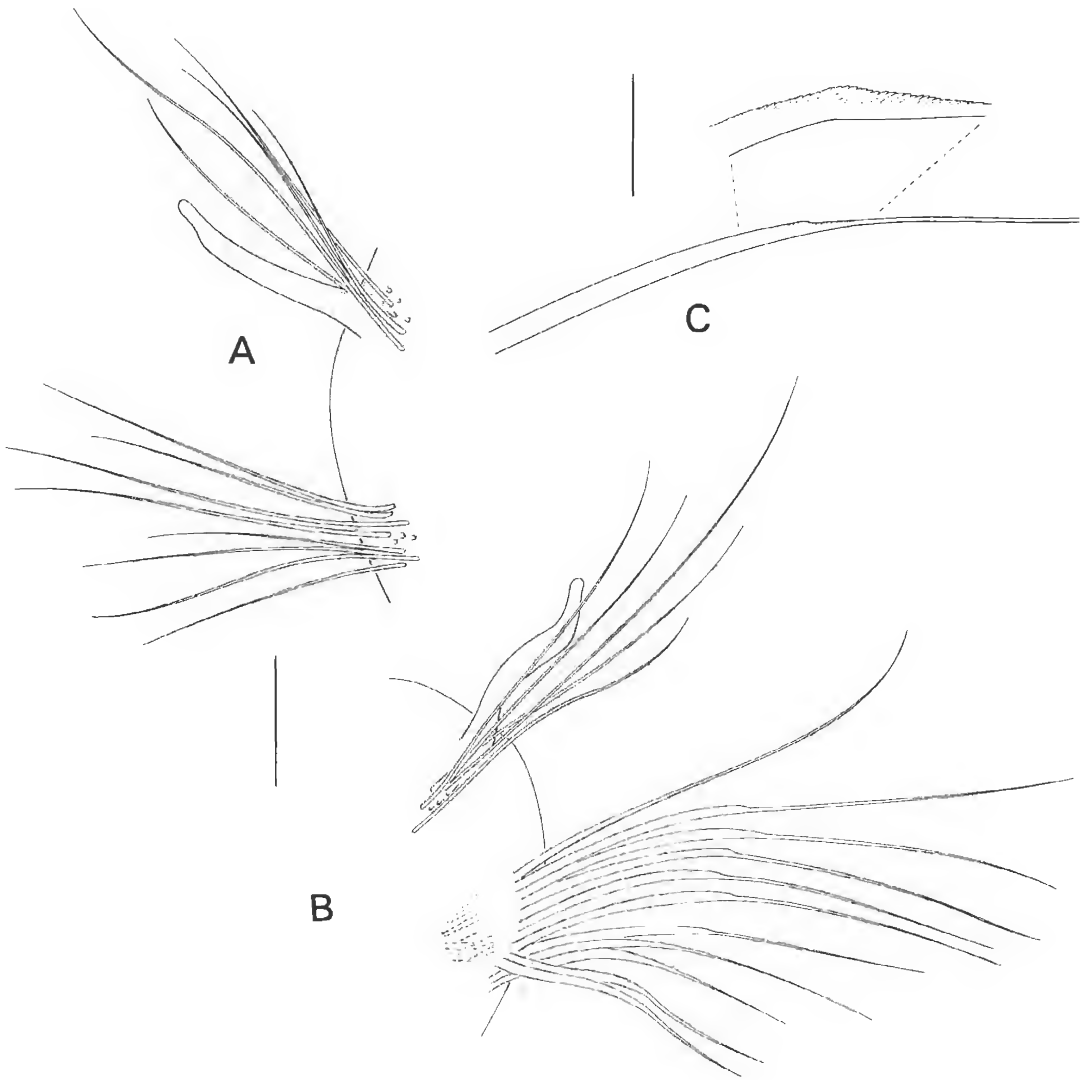

Figure 3. ***Aricidea (Allia) bryani*****, new species. Representative postbranchial parapodia, anterior views, number of setae reduced for clarity. A. Setiger 37. B. Setiger 118. C. Detail of neuroseta from setiger 118. Scales: A, B = 0.1mm; C = 0.05mm.**

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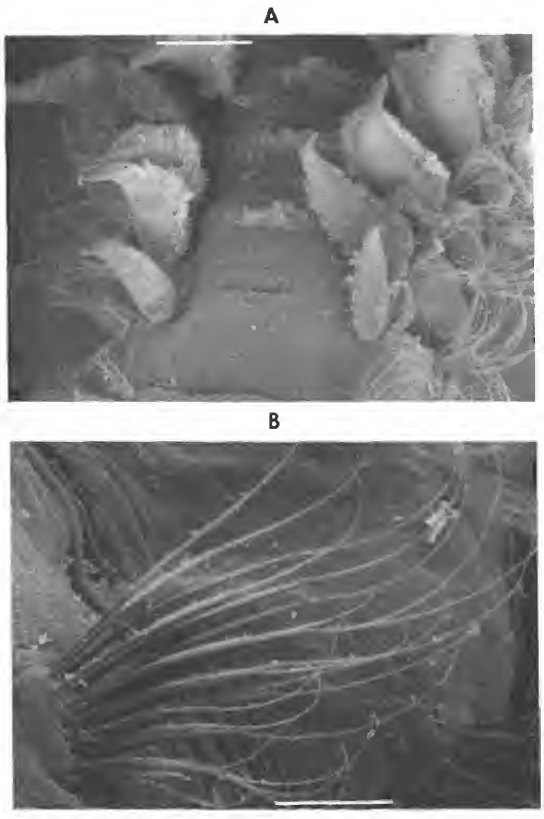

Figure 4. ***Aricidea (Allia) bryani*****, new species. Scanning electron micrographs. A. Posterior branchial region, dorsal view. B. Neuropodial fascicle from posterior region, anterior view (setiger 130). Scales: A = 0.1mm; B = 0.05mm.**

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Observations on an Isolated Population of *Sagitta hispida* Conant (Chaetognatha) in a Tropical Lagoon System of Northeast Yucatan (Mexico)

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OBSERVATIONS ON AN ISOLATED POPULATION OF *SAGITTA HISPIDA* CONANT (CHAETOGNATHA) IN A TROPICAL LAGOON SYSTEM OF NORTHEAST YUCATAN (MEXICO)

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ABSTRACT Monthly zooplankton collections were carried out from January to December 1991 at two sampling sites, Cuenca Norte and Bojórquez lagoon, in the Nichupté lagoon system, a partially enclosed network of interconnected waterways located in the northeastern region of the Yucatan Peninsula (Mexico) adjacent to the Caribbean Sea. Only one species of Chaetognatha, *Sagitta hispida* Conant, was present and was more abundant at Cuenca Norte (total density 450.6 organisms/m^3) than at Bojórquez (138.6 organisms/m^3). The latter site is smaller, more physically isolated, and more environmentally stressed than the former. From monthly gonadal and length-frequency analyses of 1390 specimens, it was found that (1) total length significantly differed among four successive maturity stages, (2) juvenile and immature specimens occurred in greater numbers at Bojórquez, while more mature specimens comprised a greater percentage of individuals found at Cuenca Norte, and (3) individuals collected at Bojórquez, where slightly higher temperatures were recorded, were significantly smaller than those from Cuenca Norte. The latter two findings indicate that *Sagitta hispida* spawns at a higher frequency at Bojórquez, possibly due to the cumulative effect of higher temperature.

Introduction

Chaetognaths are among the most abundant holoplanktonic animals in oceanic, neritic and coastal environments (King 1979; Øresland 1990). Like most other zooplankters, chaetognaths produce more generations at lower latitudes where temperatures are higher (Dunbar 1941, 1952, 1962; McLaren 1963; Alvariño 1965; Sameoto 1971). This higher breeding frequency has an impact on the composition of other zooplankters such as copepods which are reported to be their main prey item (Reeve 1970; Szyper 1978; Pearre 1980; Canino and Grant 1985; Alvarez-Cadena 1993).

Sagitta hispida Conant, 1895, is a conspicuous chaetognath commonly occurring in neritic waters on both sides of the tropical-equatorial Atlantic Ocean (Alvariño 1965). It has often been reported as abundant in coastal waters of the Gulf of Mexico (Pierce 1951; McLelland 1989), along the east coast of the United States (Pierce 1953; Pierce and Wass 1962; Owre 1960; Grant 1962, 1963) and from some areas of the Caribbean Sea (Suárez-Caabro 1955; Michel 1984; Suárez-Morales et al. 1990; McLelland and Heard 1991; McLelland et al. 1992). In tropical regions, especially in areas with dense submerged vegetation, *Sagitta hispida* has been observed with a near bottom distribution (Owre 1972; McLelland and Heard 1991) leading Bieri (1991) to consider the species as "quasi-planktonic". Other field studies demonstrate a marked diel migration for the species; mature specimens are rarely collected in surface waters during the day (McLelland and Heard 1991). Sweatt and Forward (1985) determined in laboratory studies that *S. hispida* demonstrates all-or-none upward vertical movement when ambient light intensity is below approximately $10^{16.7}$ photons $m^{-2}. s^{-1}$.

There are no previous reports on the chaetognath fauna in the Nichupté lagoon system (NLS), located on the Mexican coast of the Caribbean Sea. In this paper, we document the unique occurrence of *Sagitta hispida* in this lagoon system and compare the populations of the species at two sites with differing hydrological conditions, while considering data on body length and four preadult maturity stages.

Study Area

The NLS is located adjacent to the Caribbean Sea (21° 07'N, 86° 46'W) in the northeastern region of the Yucatan Peninsula (Figure 1). The climate in the region is subhumid and warm (lowest temperatures are higher than 18° C) with the main rainy season in summer and moderate rainfall in winter (type AW1 (X')(i') g of García 1964). Although the NLS (type IV-B of Lankford 1976) at present is largely surrounded by tourist facilities, it was originally bordered by mangrove vegetation. In tropical oligotrophic

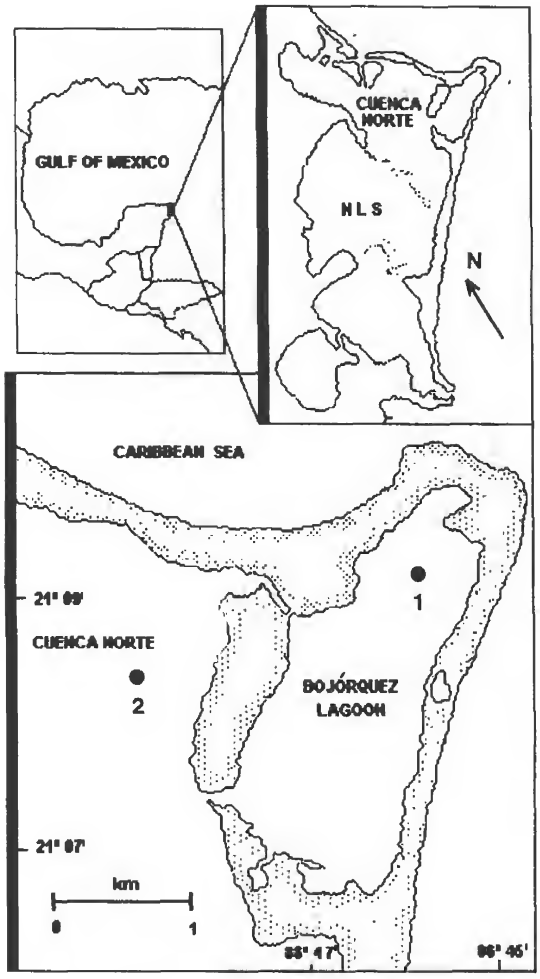

Figure 1. Study area and sampling sites in the Mexican Caribbean.

systems such as NLS, the typical submerged vegetation is characterized by *Thalassia testudinum*, *Halodule* sp. and rhyzophytic or calcareous algae. The soil in the area is highly porous and permeable (Butterlin 1958; López-Ramos 1974). Freshwater runoff from rivers that typify other lagoon systems is lacking. Subterranean springs and "cenotes" (karstic water deposits) provide variable amounts of freshwater input into the system.

Three climatic regimes are reported annually in this area: "nortes", dry, and rainy seasons (Merino and Otero 1991). During the present study period (1991), "nortes", identified by the strong northern winds which blow in the area, extended from December to March. The dry season, with dominant southeastern winds and low precipitation, extended from April to July. The rainy season, with the same wind pattern but higher precipitation, extended from August to November.

For this study, two intrinsically different sampling areas at adjacent locations within the NLS (Figure 1) were compared. Station 1 consisted of two sites in Bojórquez lagoon, a nearly enclosed, shallow (1.5 m average depth) lagoon with two narrow openings to other parts of the NLS. Bojórquez lagoon is further characterized by high salinity, nutrient enriched water from organic pollution, and patches of *Thalassia testudinum* and *Halodule*. Anthropogenic stress at this site is considerable and was detailed by Alvarez-Cadena and Segura-Puertas (in preparation). Station 2 was located in Cuenca Norte, a larger, less stressed body of water averaging 2.5 m in depth and with a small, but distant opening to the Caribbean Sea to the north. Of the two, station 2 is, on the whole, more representative of the NLS.

MATERIALS AND METHODS

Zooplankton samples and surface hydrographic data (salinity and temperature) were collected at the two stations monthly from January to December 1991. All collecting was performed between 0900 and 1100 hrs. A conical plankton net (0.42 m diameter, 330 μm mesh) was equipped with a General Oceanics flowmeter and towed in a circular path near the surface for 5 minutes at 1.5 knots. Samples were preserved in the field with buffered (lithium carbonate) formalin at a concentration of 5% formalin-seawater. In the laboratory, all chaetognaths collected were counted and examined for species identification, whereupon at least 50 animals were randomly removed from each sample, measured to the nearest 0.1 mm, and examined for gonadal condition. Measurements were made from the tip of the head to the tail excluding the tail fin. Maturation stages were assigned as follows and were based on various classifications reviewed by Alvariño (1965):

Stage I. Ovaries not visible, or if present are very small, not reaching anterior part of posterior fin; few small ovocites present. Seminal vesicles not present and no sperm visible in tail segment.

Stage II. Ovaries visible, usually reaching mid space between lateral fins or posterior part of anterior fin; small ovocites present. Some thickening occurring near end of posterior fin indicating primordium of seminal vesicles; sperm present but not occupying entire tail segment.

Stage III. Ovaries reaching midpoint of anterior fin; ovocites rounded. Seminal vesicles visible but somewhat flattened; sperm present throughout tail segment.

Stage IV. Ovaries reaching anterior part of anterior fin; ovocites rounded, arranged usually in two rows. Seminal vesicles oval to rectangular, testes full of sperm.

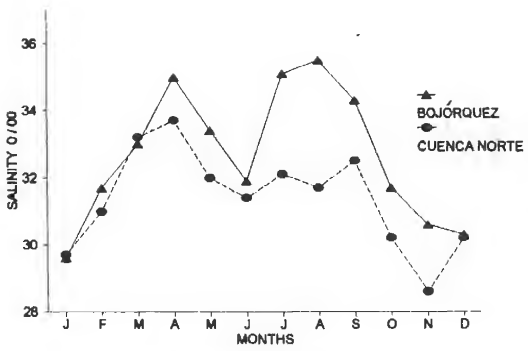

Figure 2. Annual variation of salinity at Bojórquez and Cuenca Norte. Bojórquez data are mean values recorded from two sampling sites.

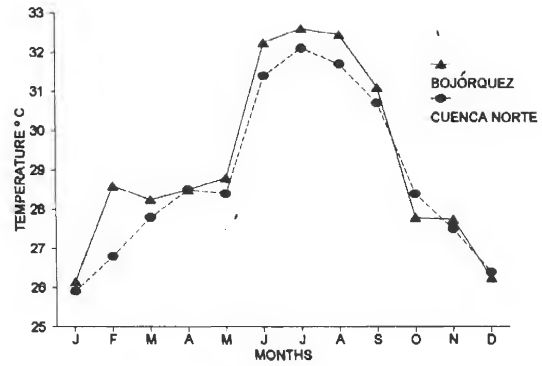

Figure 3. Annual variation of temperature at Bojórquez and Cuenca Norte. Bojórquez data are mean values recorded from two sampling sites.

Stage V. Ovaries reaching posterior part of ventral ganglion, ovocites fully matured. Seminal vesicles completely full or sometimes "spent" (empty).

Chaetognath data from the two sites in Bojórquez were pooled as the mean lengths of the animals did not show any significant differences. Likewise, pooled values are given for salinity and temperature recorded from Bojórquez (Figures 2,3).

Data from the two stations were compared statistically using mean length measurements (total N=1390) for maturity stages I-IV. A two-way analysis of variance (ANOVA) was used to test for variation among stages at each station and for differences between stations. A t-test was employed to further compare the differences among the mean lengths of each maturity stage between the two stations. Testing was performed at α=0.05 with confidence intervals of 99%.

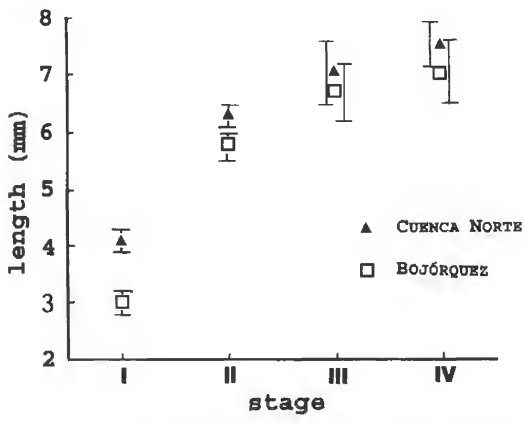

Figure 4. Annual mean length variations of *Sagitta hispida* maturity stages I-IV at Cuenca Norte and Bojórquez.

RESULTS

Salinity at Bojórquez was usually higher than at Cuenca Norte (Figure 2). Lower salinity was observed at both stations during January ("nortes") with 29.63 and 19.72‰ respectively. Highest values were recorded in August for Bojórquez (35.44‰) and in July for Cuenca Norte (34.68‰). Temperature was usually slightly higher at Bojórquez than at Cuenca Norte (Figure 3). At both stations, the lowest temperature was recorded in January and the highest in July-August.

The taxonomic analysis of chaetognaths collected at both sites during the survey revealed the presence of only one species, *Sagitta hispida*. It was about three times as abundant at Cuenca Norte (450.6 organisms/m³) than at Bojórquez with a total density of 138.6 organisms/m³ (Table 1). Proportionally, stage I was the most common of all maturity stages, and comprised a higher percentage of the population at Bojórquez (89.2%) than at Cuenca Norte (71.2%). Stages II to IV were consistently higher in percentage at Cuenca Norte than at Bojórquez (14.1% stage II, 6.4% stage III, and 8.3% stage IV from Cuenca Norte and 7.6% stage II, 1.8% stage III, and 1.4% stage IV for Bojórquez). Only seven adult specimens of stage V (8.5-9.0 mm total length) were collected during the survey, six at Cuenca Norte and one at Bojórquez. Because of this extreme scarcity, comparative analyses were omitted for this stage.

Mean lengths were variable during the annual cycle (Table 2, Figures 4, 5a and 5b), especially those of stage I at Bojórquez which ranged from 1.59 mm in August to > 4 mm in March, April and November (variation range = 64%). Stage I animals at Cuenca Norte did not show this range of size variability (3.18 mm in May to 4.8 mm in August; variation range 33%). The mean lengths of the remaining stages showed less variability at both sampling areas.

TABLE 1

Numbers analyzed monthly for maturity stages I-IV and total density (organisms/m³) of *Sagitta hispida* at Bojórquez and Cuenca Norte. Data for Bojórquez represent pooled values from two sites.

Bojórquez

Stage	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total (org./m³)	%
I	49	82	41	16	70	80	82	78	98	72	40	18	726	89.2
II	0	8	8	9	1	2	1	13	2	13	5	0	62	7.6
III	0	0	0	2	1	0	1	5	0	3	3	0	15	1.8
IV	1	0	1	1	0	0	0	4	0	2	2	0	11	1.4
Org./m3	11	5.9	3.1	2.1	4.1	15.3	5.1	39.5	19	13.5	13	6.3	138.6	-

Cuenca Norte

Stage	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total (org./m³)	%
I	29	30	38	39	21	37	42	18	35	44	35	42	410	71.2
II	11	12	8	8	0	7	1	9	7	3	7	8	81	14.1
III	5	6	2	2	0	2	4	6	4	1	5	0	37	6.4
IV	5	2	2	1	0	4	3	17	9	2	3	0	48	8.3
Org./m3	5	9.6	12.5	15.8	44.3	82.5	49.4	133.3	17.9	0.3	31	49	450.6	-

Length data for each stage at the two sites (1390 measurements) were tested using a two-way analysis of variance (ANOVA) for variation in mean length in sampling sites vs. the four maturity stages. Residual data showed a normal distribution with the Kolmogorov-Smirnov test. It was found that significant differences ($\alpha = 0.05$) existed in length vs. sampling site ($F_s=9.55, p=0.002$), maturity stage ($F_s=227.97$, $p<0.001$), and interaction between the two factors ($F_s=13.12, p<0.001$). It was further found that total length significantly differed among successive maturity stages and that individuals collected at Cuenca Norte were significantly larger than those caught at Bojórquez for each of the four maturity stages (Figure 4). Both parameters were constant at all levels and the terms of significant interaction were not evident, but could be explained by the larger difference between stage I lengths at both sampling sites (Figure 5a,b).

In order to compare mean length differences of each maturity stage between the two sampling sites, t-tests were used with confidence intervals of 99% and testing HO at $\alpha=0.05$. For stage I, with 1136 individual length measurements (410 from Cuenca Norte and 726 from Bojórquez), differences were highly significant ($t=-17.58$), exhibiting the widest range of difference between the two sampling areas. For stage II, with less available data ($N=143$), differences were also significant ($t=-3.46$). However, the same analyses with stage III and IV data revealed no differences at 99% C.I. between the lengths of Cuenca Norte and Bojórquez specimens. Finally, when this analysis was performed pooling the total number of observations (1390) from all stages at each station group, the differences were found to be significant ($t=18.58$) between the two sampling areas.

TABLE 2

Monthly mean length (mm) of *Sagitta hispida* for maturity stages I-IV at Bojórquez and Cuenca Norte. Data for Bojórquez represent pooled values from two sites.

Bojorquez

Stage	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Grand Mean
I	2.61	2.77	4.17	4.72	3.32	2.62	2.17	1.59	2.98	2.53	4.36	3.34	2.956
II	---	5.66	5.87	6.44	5.70	---	---	---	---	---	5.56	---	5.761
III	---	---	---	7.00	5.50	---	---	---	---	---	6.76	---	6.660
IV	7.45	---	6.90	---	---	---	---	---	---	---	8.00	---	7.070

Cuenca Norte

Stage	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Grand Mean
I	4.27	4.72	4.28	4.14	3.18	3.21	3.68	4.77	4.46	4.23	4.46	3.34	4.181
II	6.10	6.84	6.21	6.01	---	5.13	6.00	6.61	6.02	6.40	6.01	---	6.251
III	7.48	7.80	6.70	6.90	---	6.00	6.65	6.98	7.02	6.70	6.40	---	6.985
IV	8.10	7.25	7.76	7.70	---	6.54	7.13	7.34	7.57	7.70	7.76	---	7.481

DISCUSSION

Identification of immature chaetognaths is often difficult and confusing because of their small size and undeveloped, taxonomically important sexual features. This is particularly complicated in localities where several species co-occur. *Sagitta hispida*, however, can be readily identified by its conspicuously large, wide head, square to bean-shaped eye pigment and the presence of gut diverticulae in larger specimens (McLelland 1989). Immature specimens can be distinguished from similar species by examining the anterior teeth which, under high magnification, appear to lie flat against the head, their tips forming a line nearly perpendicular to the body axis; furthermore, the teeth have longitudinal ridges which give them a quadrangular cross-sectional appearance. These ridges can be seen in SEM photographs by Cosper and Reeve (1970). In mature specimens, the ovaries often extend past the midpoint of the anterior fins and contain round ova arranged in two rows. The lateral fins are completely rayed, with the posterior fins reaching the seminal vesicles. The latter are oval to rectangular when ripe, and separated from the caudal fin by half their length.

Sagitta hispida has been reported to tolerate wide ranges of salinity and temperature. Experiments by Reeve and Walter (1972) on growth rates in laboratory populations found that the species will not survive more than 15 days at temperatures above 33.5°C. They also found that the species can grow to maturity in salinity ranges from 25-40‰, but failed to mature at 20‰ and below. At both stations in the NLS, temperatures were never below 25°C or above 33°C. On the other hand, salinities were not below 28‰ or above 36‰. Thus, the ranges were well within the known ranges of growth and maturation and may explain the local success of *S. hispida*. The fact that the species was represented in our samples mainly by immature specimens likely indicates a combination of continuous spawning of the population, a greater mortality of older individuals, and the known differential vertical distribution of juvenile and adult chaetognaths. *Sagitta hispida* is known to breed more or less continuously throughout the year in tropical and subtropical waters (Pierce 1951; Owre 1960), resulting in a large proportion of immature specimens representing multiple generations coexisting at different stages of development (Pearre 1991). A concentration of immature chaetognaths in the upper water column is thought to be an indication of shallow-water spawning (Stone 1969) or better survival conditions for younger individuals (Raymont

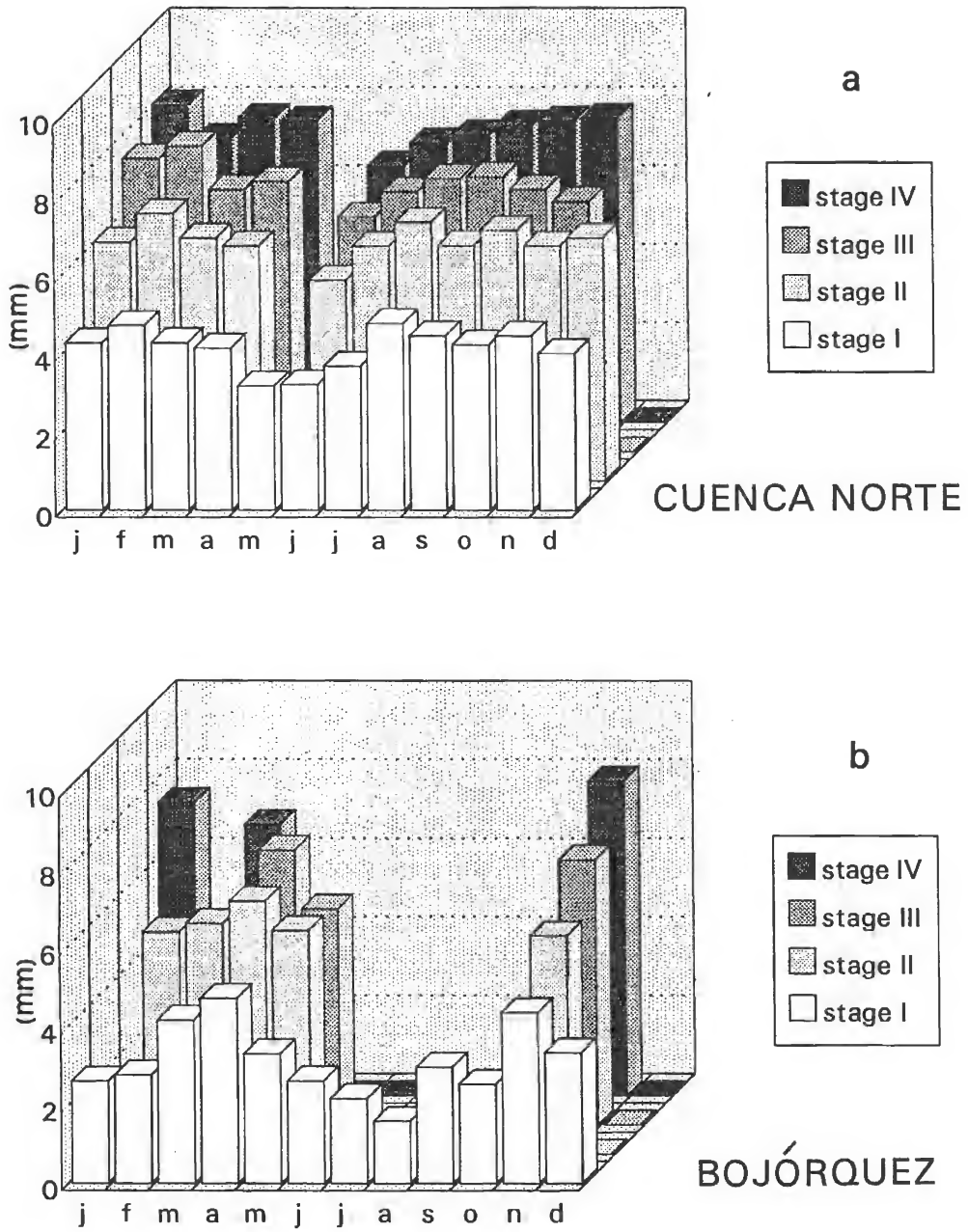

Figure 5. Monthly mean length of *Sagitta hispida* maturity stages I-IV at Cuenca Norte and Bojórquez.

1963; McLelland 1984). Adults of *S. hispida* have been reported to dwell near the bottom or associate with submerged seagrass beds during the day (Owre 1972; Sweatt and Forward 1985; McLelland and Heard 1991) and migrate upward at night. Although our sampling efforts were near the surface, our data confirms that young individuals can be found abundantly in the water column even during the day. In additional samples (not reported here) collected over a 24-hour period in April and October in Bojórquez lagoon, larger numbers of adult animals were collected at night, further supporting the assumption of diel migration for this species.

The statistical analyses of length variations in the surveyed populations of *Sagitta hispida* clearly showed that (1) successive maturity stages are well defined in regards to corresponding body length, (2) differences between sampling stations were more apparent in stages I and II, and (3) Cuenca Norte individuals were consistently larger at all stages as compared to those from Bojórquez. Food availability did not seem to be the reason for differences in size between the two stations. In a concurrent study, Alvarez-Cadena and Segura-Puertas (in preparation) found that copepods in Bojórquez, where chaetognaths were smaller, were nearly three times as abundant as those of Cuenca Norte (6177.6 and 2236/m^3 respectively, annual abundance). On the other hand, it is possible that when food is overly abundant, the metabolic energy of the chaetognath is shifted to reproductive output instead of growth, accounting for a smaller size at maturity (faster rate of maturation) at Bojórquez. The two dominant genera of the NLS copepod population, *Acartia* and *Paracalanus*, were reported by Reeve (1966) to be the main food items for *S. hispida* in Biscayne Bay, Florida.

Temperature differences between the two stations might account for the observed dissimilarities in body sizes and relative proportions of maturity stages. For some zooplanktonic organisms, temperature seems to be related not only to the attainment of larger size at maturity, but also with the number of generations produced by the species. Dunbar (1941) mentioned that "it is generally true that zooplankters of high latitudes (colder waters) develop more slowly, reach larger size and live longer than related forms in warmer areas." McLaren (1963, 1966) reported that *Sagitta elegans*, a circumboreal species, required more time to reach maturity at lower temperatures. It may be argued that temperature differences between Bojórquez and Cuenca Norte are very small (on the order of 1°C or less) and not significant enough for these differences. However, the cumulative effect of temperature, rather than just the slightly higher values, may be responsible for the smaller mean size yet apparently higher spawning frequency of *S. hispida* at Bojórquez. Sameoto (1971) reported that once *S. elegans* had accumulated 738°C degree-days, the species would reach maturity. Jakobsen (1971) remarked that small differences in temperature could promote distinct gonadal development due to the extent of the period they exerted their influence. Dunbar (1962) also found that although hydrographic differences (e.g., temperature) were not large, they also had a cumulative effect on the biology of *S. elegans*.

Reeve and Walter (1972) reported that *Sagitta hispida* completes its entire life cycle in 18 to 50 days. Although the number of generations for *S. hispida* cannot be accurately determined in this work because samples were collected at monthly intervals, apparently a higher number of generations is produced at Bojórquez than at Cuenca Norte as evidenced by the higher frequency of juveniles (stage I) recorded throughout the year. A more frequent sampling effort might clarify this.

The population of *Sagitta hispida* in the NLS, especially Bojórquez, is largely isolated from continual genetic input from coastal populations by the combined effects of low-energy tidal flow and poor circulation which hinder exchange with the adjacent marine environment. Residence of water in the NLS, as a whole, has been estimated at about two years (Merino et al. 1990), except for occasional catastrophic events such as Hurricane Gilbert in 1988 which overwashed the narrow land barrier separating the lagoon system from the Caribbean Sea. In Bojórquez, wind-aided circulation has been further diminished by the dense line of hotels and other tourist facilities along the Caribbean seacoast which obstruct the dominant southeast trade winds from reaching the lagoon. In a personal communication to the second author, S. Van der Spoel suggested that tangible variations begin to appear in a typical planktonic species population that has been isolated for 200 generations. Given a more or less continual rate of growth and a generation turnover of 20-30 days for *Sagitta hispida*, such population divergences should occur within 10-15 year intervals. Thus, it is possible that because of its isolation, a population of *Sagitta hispida* characterized by smaller adults is evolving in Bojórquez lagoon.

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Distribution of *Loxothylacus texanus* (Cirripedia: Rhizocephala) Parasitizing Crabs of the Genus *Callinectes* in the Southwestern Gulf of Mexico

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DISTRIBUTION OF *LOXOTHYLACUS TEXANUS* (CIRRIPEDIA: RHIZOCEPHALA) PARASITIZING CRABS OF THE GENUS *CALLINECTES* IN THE SOUTHWESTERN GULF OF MEXICO

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ABSTRACT A preliminary study on the interaction between the parasitic barnacle *Loxothylacus texanus* and two of its host species, the blue crab *Callinectes sapidus* and the dark blue crab *C. rathbunae*, in the Gulf of Mexico is presented. Data were obtained from 923 crabs, 162 *C. sapidus* and 761 *C. rathbunae*, deposited in the Colección de Crustáceos, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), that were collected in 14 coastal lagoons and sites along the Mexican coast of the Gulf of Mexico. The distribution of *L. texanus* parasitizing each one of the host species, mean host size variation, distribution of number of parasite externae per host, and morphological modifications of the abdomen of the hosts are analyzed.

Introduction

The crabs of the genus *Callinectes*, mainly the blue crab *C. sapidus* Rathbun, and the dark blue crab *C. rathbunae* Contreras, support one of the most important commercial fisheries, both in terms of volume and value, within the Gulf of Mexico (Anonymous 1994). Of the biotic factors that affect blue crab populations negatively in the Gulf of Mexico, the parasitism by rhizocephalan barnacles may be one of the most important, periodically reaching very high prevalences (Wardle and Tirpak 1991; Lorán et al. 1993).

Rhizocephalan barnacles parasitize susceptible shrimps and crabs through a planktonic larval stage from which an endoparasitic phase originates, a phase that is not evident unless the host is examined histologically. During the internal phase of the parasite, the host external morphology changes; the male abdomen becomes broader through a process that has been called feminization (Reinhard 1950). The emergence of a reproductive body called "externa" follows the endoparasitic phase. The externa emerges through the internal surface of the abdomen of the host after molting, while the host exoskeleton is still soft. In host species parasitized by the rhizocephalan family Sacculinidae, hosts will not molt once the externa has appeared and the mean size of parasitized hosts is usually significantly less than that of unparasitized hosts (Reinhard 1956; O'Brien and Van Wyk 1984). However, the most important effect caused by this parasitism is that host gonads do not mature (parasitic castration *sensu* O'Brien and Van Wyk 1984). The effects of the parasitism by rhizocephalans at the population level are: a) parasitized individuals are not removed by the commercial fishery from the population because they do not attain legal size, and b) the parasitized fraction, which does not reproduce, competes with unparasitized individuals for food and space.

Studies have been carried out on the distribution of rhizocephalans (Hochberg et al. 1992), host size distribution (Christmas 1969; Adkins 1972; Ragan and Matherne 1974), changes in prevalence during outbreaks (Christmas 1969; Park 1969; Wardle and Tirpak 1991), the relationship between parasite size (externa size) and host size (Reinhard 1950; Wardle and Tirpak 1991), and the morphological changes that parasitized crabs undergo (Reinhard 1950; Hochberg et al. 1992). In spite of the great economic importance of the blue crab fishery in Mexico, only two studies have recorded data on the prevalence of *Loxothylacus texanus* Boschma, its seasonal variation, and host size variation: Lorán et al. (1993), who analyzed the crab populations of Alvarado lagoon and Lázaro-Chávez et al. (in press), who studied parasitized crabs in Tamiahua lagoon. The objective of this study is to present additional records of parasitized crabs of the genus *Callinectes* within the Gulf of Mexico in order to update the known distribution of *L. texanus*, to determine what host species are being parasitized, to establish the host size range, and to present figures of the most common type of morphological variations of parasitized crabs.

Materials and Methods

Data on parasitized blue crabs from the southwestern Gulf of Mexico were obtained through the examination of all the *C. sapidus* and *C. rathbunae* from the Gulf of Mexico deposited in the Colección de Crustáceos, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

Fourteen localities were represented in these samples, including coastal areas and coastal lagoons (Altamira, Chairel, Pueblo Viejo, Tamiahua, Casitas, La Mancha, Mandinga, Alvarado, Sontecomapan, Coatzacoalcos, Machona, Atasta, Términos, and Champotón) as depicted in Figure 1. Detailed descriptions of these coastal lagoons and sites can be found elsewhere in a number of papers (Contreras 1985; Yáñez-Arancibia and Day 1988; Rosas 1989). Each crab was identified and examined for the presence of rhizocephalan externae. All crabs were sexed and the shape of the abdomen recorded; sex was determined through the inspection of gonopods and genital pores. In this way, feminized crabs (crabs that are parasitized but which do not yet show the parasite externa) were also found. The internal surface of the abdomen of all crabs was examined in search of small externae or scars of *Loxothylacus texanus* and the number of externae per crab was recorded. The distribution of number of parasite externae per host was compared to a Poisson (random) distribution with a chi-square test. The most common types of abdomens of parasitized male crabs were identified (triangular and rounded) and parasitized individuals were classified accordingly by species. A G-test of independence was used on a 2 x 2 contingency table, to test if triangular abdomens were equally frequent in males of both host species, and a Student's t-test was used to compare their mean sizes. The two types of abdomens for parasitized males and the extra broad abdomen of parasitized females were figured to aid in their identification in the field. Crab sizes correspond to carapace width in millimeters (mm), and mean values are followed ± one standard error.

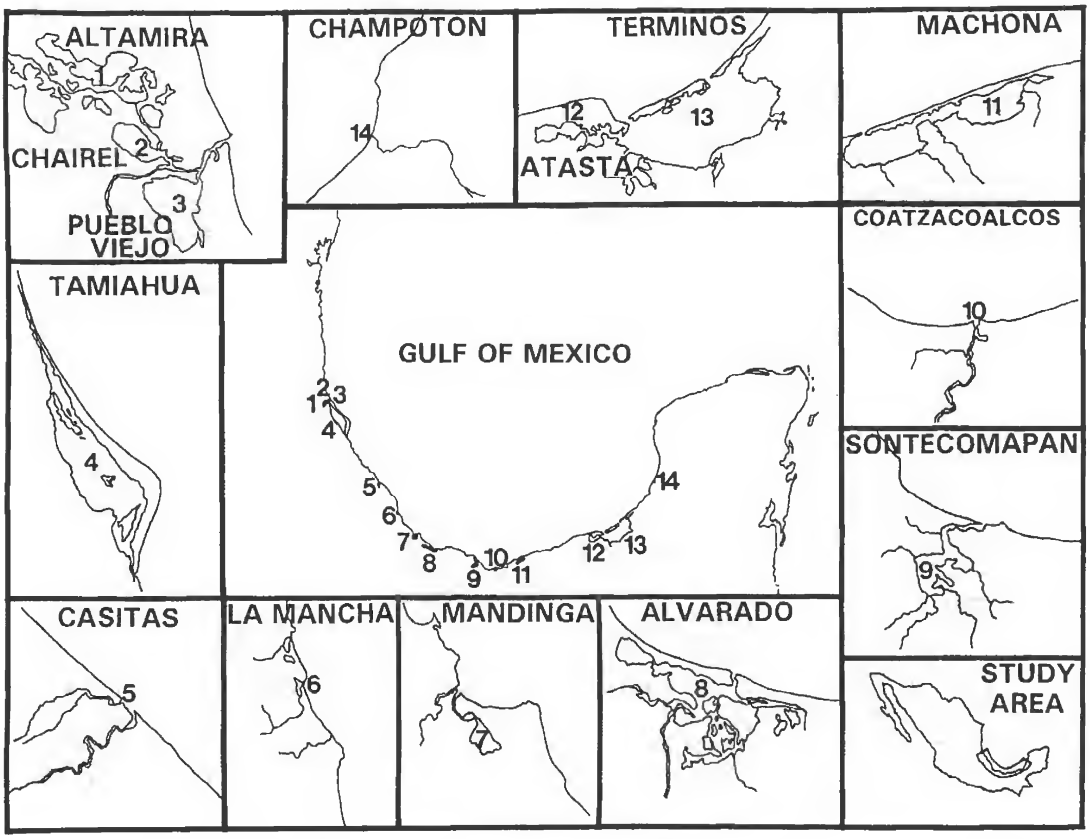

Figure 1. Collection sites in the Gulf of Mexico of *Callinectes sapidus* and *C. rathbunae*.

TABLE 1

Samples of crabs examined by species and locality within the Gulf of Mexico. Values represent number of parasitized crabs/ number of unparasitized crabs, and mean host size/mean size of unparasitized crabs, followed by ± one standard error. Asterisks represent significant differences between means. All material is deposited in the Instituto de Biología, UNAM.

	Callinectes sapidus		*Callinectes rathbunae*	
Altamira, Tamaulipas	0/9			
Chairel, Veracruz			0/21	
Pueblo Viejo, Veracruz	5/1	87.8 ± 10.4	0/90	
Tamiahua, Veracruz	9/53	123.0 ± 3.7 / 116.4 ± 2.5	0/289	
Casitas, Veracruz			1/4	101.4
La Mancha, Veracruz	0/13		0/5	
Mandinga, Veracruz	0/5		6/123	120.2 ± 7.2 / 109.9 ± 1.5
Alvarado, Veracruz	0/4		0/112	
Sontecomapan, Veracruz	24/32	91.6 ± 1.9 / 111.2 ± 3.0*	67/26	86.0 ± 1.6 / 104 ± 3.3*
Coatzacoalcos, Veracruz			0/2	
Machona, Tabasco			1/0	94.3
Atasta, Campeche			1/0	75.2
Términos, Campeche	0/7		7/5	81.9 ± 3.3 / 113.5 ± 3.8*
Champotón, Campeche			0/1	

* P< 0.001

RESULTS

A total of 923 crabs, 162 *C. sapidus* and 761 *C. rathbunae*, was examined; from this total, 38 *C. sapidus* (23.5%) and 83 *C. rathbunae* (10.9%) were parasitized (Table 1). Parasitized crabs were found in eight of the 14 coastal lagoons and sites that were examined (Pueblo Viejo, Tamiahua, Casitas, Mandinga, Sontecomapan, Machona, Atasta, and Términos) as shown in Table 1.

In our samples, *C. sapidus* was parasitized in Pueblo Viejo, Tamiahua, and Sontecomapan lagoons, Veracruz. The mean size of parasitized *C. sapidus* ranged from 87.8 ± 10.4 mm in Pueblo Viejo lagoon to 123.0 ± 3.7 mm in Tamiahua lagoon; the range of sizes of parasitized *C. sapidus* was from 70.0 to 134.6 mm, both from Tamiahua lagoon, Veracruz. *C. rathbunae* was parasitized in Casitas and the coastal lagoons of Mandinga and Sontecomapan, Veracruz; Machona lagoon, Tabasco; and Atasta and Términos lagoons, Campeche. The mean size of parasitized *C. rathbunae* ranged from 81.9 ± 3.3 mm in Términos lagoon, Campeche, to 120.2 ± 7.2 mm in Mandinga lagoon, Veracruz; the range of sizes of parasitized *C. rathbunae* went from 45.3 mm in Sontecomapan lagoon, Veracruz, to 144.1 mm in Mandinga lagoon, Veracruz. In Términos lagoon, parasitized *C. rathbunae* and parasitized crabs of both species in Sontecomapan lagoon were significantly smaller than unparasitized crabs; while there were no significant differences in the rest of the localities.

The number of parasite externae in *C. sapidus* varied from one to three; 32 crabs had one externa (84.2%), five had two (13.2%), and one had three (2.6%). In turn, in *C. rathbunae*, the number of parasite externae ranged from one to four; 50 crabs had one parasite externa (60.2%), 20 had two (24.7%), 11 had three (13.6%), and two had four(2.5%). Assuming that the distribution of parasites is the result of similar processes in all the sites studied and since these distributions depart considerably from normality, the distribution of number of externae per host was analyzed only by species and not by locality. In *C. sapidus*, the distribution of externae corresponds to a random distribution (Table 2), while in *C. rathbunae*, the distribution departs considerably from random and approaches a contagious one, with a larger proportion of hosts having multiple externae (Table 3).

Out of 38 parasitized *C. sapidus*, 11 (28.9%) were males and 27 (71.1%) were females; while in *C. rathbunae*, there were 41 parasitized males and 42 parasitized females. In *C. sapidus*, three of 11 males (27.3%) had a triangular abdomen, while the remaining eight had a broad abdomen. For *C. rathbunae*, nine out of 41 crabs (22%) had a triangular abdomen (Figure 2). The frequency of appearance of triangular abdomens was independent of the host species

TABLE 2

Distribution of externae of *Loxothylacus texanus* on 162 *Callinectes sapidus*. Observed frequencies are compared (Chi-square test) to the expected frequencies of a Poisson (random) distribution.

No. externae per host	Observed frequencies	Expected frequencies	$(O-E)^2/E$
0	124	122.72	0.013
1	32	34.11	0.130
2	5	4.74	0.014
3	1	0.44	0.712
Total	162	162.01	χ^2=0.869, P>0.05

TABLE 3

Distribution of externae of *Loxothylacus texanus* on 761 *Callinectes rathbunae*. Observed frequencies are compared (Chi-square test) to the expected frequencies of a Poisson (random) distribution.

No. externae per host	Observed frequencies	Expected frequencies	$(O-E)^2/E$
0	678	640.57	2.18
1	50	110.17	32.86
2	20	9.47	11.70
3	11	0.54	202.61
4	2	0.02	169.93
Total	761	760.77	χ^2=419.30, P<0.0001

(χ^2[1]=1.83, P>0.05); in other words, triangular abdomens are equally frequent in both host species. Mean host sizes for males with triangular abdomen were 83.76 ± 8.29 mm (range 67.2 to 92.7 mm) for *C. sapidus* and 82.25 ± 6.53 mm (range 45.3 to 115.8 mm) for *C. rathbunae*; no significant differences were found between the two mean values (t-test, t[10]=0.121, P>0.05).

DISCUSSION

Two species of the genus *Callinectes*, *C. sapidus* and *C. rathbunae*, are parasitized by *Loxothylacus texanus* in the southwestern Gulf of Mexico. *C. sapidus* is parasitized, as indicated by the records presented here and complemented with the information provided by Lorán et al. (1993), throughout the coast of the State of Veracruz from Tamiahua lagoon south to Alvarado and Sontecomapan lagoons. No clear pattern of variation of host size can be discerned in *C. sapidus* along the Mexican coast, contrary to what Hochberg et al. (1992) found for the northern and eastern Gulf of Mexico.

While in one previous report (Lorán et al., 1993) *C. rathbunae* was found to be a second host for *L. texanus*, no information on the extent of the distribution of this association was available prior to this report. *C. rathbunae* is an endemic of the Gulf of Mexico, occurring south from the United States-Mexico border to probably Términos lagoon, Campeche (Williams 1974); however, it is parasitized only southwards from Casitas, Veracruz, to Campeche. It is relevant to note that although very large samples of *C. rathbunae* have been obtained from Tamiahua lagoon (Lázaro-Chávez et al. in press), this species has never been found parasitized in that area, confirming that it is parasitized only in the southern portion of its range. The size range of parasitized *C. rathbunae* (45.3 to 144.1 mm) is greater than that for *C. sapidus* (70.0 to 134.6 mm), and no defined pattern of host size variation along a geographic gradient is evident with the available data.

The number of *L. texanus* externae appearing in the two host species differed statistically. In *C. sapidus*, the occurrence of externae was not significantly different from a random distribution, indicating that the chances of becoming parasitized are the same for all individuals. However, in *C. rathbunae*, the number of externae per host approached a contagious distribution, suggesting that this species may occur naturally in a more aggregated pattern that may favor multiple infections (Hoeg 1982).

The recognition of parasitized crabs in the field is based on the presence of externae of the parasite and on the identification of aberrant forms of the abdomen. In this study, two types of abdomens were recognized for parasitized males: rounded, similar to a mature female abdomen, and triangular, such as those of immature females. The recognition of the two types of abdomens for parasitized males was first made by Reinhard (1950) with blue crabs from Galveston Bay, Texas. Parasitized males with triangular abdomens may not occur in all populations, as the existence of two morphologies for abdomens of parasitized males was not discussed in an investigation of parasitized blue crabs from the west coast of Florida (Hochberg et al. 1992). The frequency of appearance of both forms in males and mean size of crabs bearing a triangular abdomen did not differ significantly between host species. The origin of the triangular abdomen may be related to the number of times an infected host molts between the time of infection and the time of emergence of the externa (Alvarez 1993), so the extent of feminization would be related to the duration of the internal phase of the parasite. Consequently, the appearance of the two types of abdomens could vary seasonally and geographically.

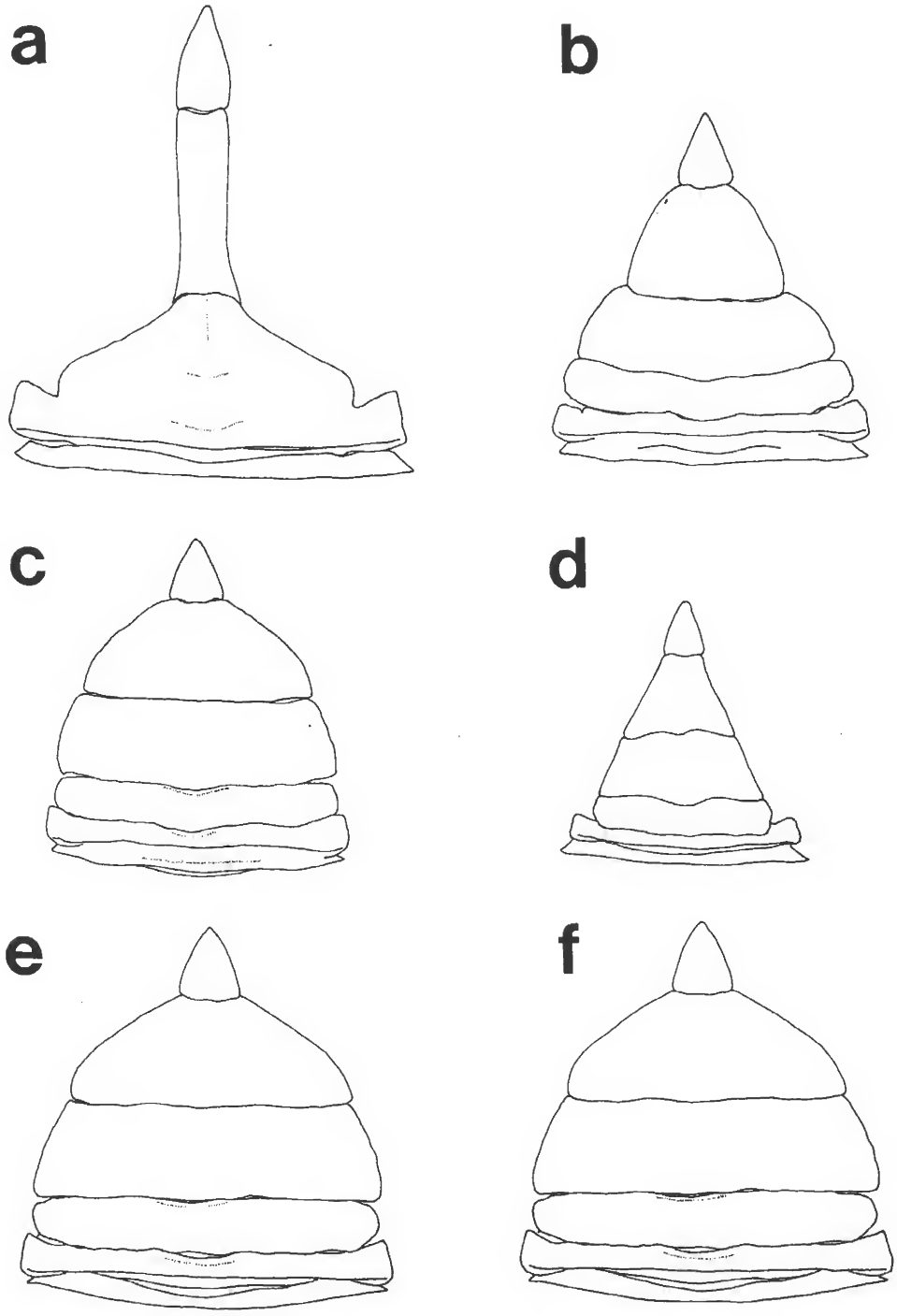

Figure 2. Abdomens of *Callinectes rathbunae*: a) normal male, b) parasitized male with triangular abdomen, c) parasitized male with rounded abdomen, d) immature female, e) normal mature female, and f) parasitized female.

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Cholangioma in a Wild-Caught Sheepshead Minnow (*Cyprinodon variegatus*) from the Northern Gulf of Mexico

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CHOLANGIOMA IN A WILD-CAUGHT SHEEPSHEAD MINNOW *(CYPRINODON VARIEGATUS)* FROM THE NORTHERN GULF OF MEXICO

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ABSTRACT A single case of a cholangioma occurred in the liver of a wild-caught sheepshead minnow (*Cyprinodon variegatus*). This is the first biliary neoplasm and second case of a hepatic neoplasm reported from a wild-caught speciment of this species. The findings further demonstrate the susceptibility of the sheepshead minnow to neoplasm development and add support to its selection as a subject for field monitoring of carcinogenic exposure.

Introduction

The use of fish species as sentinels of carcinogen exposure in the aquatic environment is a significant tool for identifying compromised ecosystems. Many important attributes of fish sentinels make their use in experimental carcinogenicity testing and field monitoring both efficacious and advantageous (Dawe and Couch 1984). One important consideration, particularly regarding field monitoring for environmental carcinogens, is the relatively low rate of spontaneous neoplasm development in fishes. Furthermore, a high correlation has been demonstrated between environmental contamination and most reported epizootics of hepatic neoplasms in fishes (Harshbarger and Clark 1990; Baumann 1992). These factors emphasize the importance of hepatic neoplasms in non-treated fishes and, particularly, in wild populations.

The sheepshead minnow, *Cyprinodon variegatus* Lacépède, has demonstrated its susceptibility to chemically-induced hepatic neoplasm development in various carcinogen studies (e.g., Couch and Courtney 1987; Hawkins et al. 1991). It is a small estuarine teleost with a limited home range inhabiting coastal waters from New England to northern South America. To date, the only reports of spontaneous neoplasm development in sheepshead minnows involve thyroid adenomas in aquarium-held specimens (Nigrelli 1952; Lightner and Meineke 1979) and a single case of hepatocellular adenoma in a wild-caught specimen (Oliveira et al. 1994). This paper describes a cholangioma found in a wild-caught sheepshead minnow from an ongoing study on P-glycoprotein expression in tissues of teleost fishes and the possible role of xenobiotics in the disruption of its function as a transepithelial efflux pump (Hemmer and Courtney, personal communication).

Materials and Methods

Approximately 30 sheepshead minnows (*Cyprinodon variegatus*) were collected in a lagoon off Santa Rosa Sound on the north shore of Santa Rosa Island, Florida, approximately 2 kilometers east of the Navarre Beach bridge. Specimens were collected by seine net, cut ventrally to open the visceral mass and immersed in Bouin's solution in the field. They were fixed for 48 hours, washed in running water for six hours and stored in 70% ethanol at room temperature. Liver, intestine and kidney tissues were dissected from 10 of the preserved specimens, dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin. Sections were cut on a rotary microtome at 5 µm, mounted on poly-L-lysine coated slides and air dried. Initial sections were processed for immunohistochemical labeling with four different P-glycoprotein antibodies [monoclonals C219, C494 and JSB-1; polyclonal mdr(Ab-1)] and counterstained with Mayer's hematoxylin (Hemmer et al. 1995). Additional sections were stained with Harris' hematoxylin and eosin.

Results and Discussion

During evaluation of samples for P-glycoprotein antibody reactions, a neoplastic lesion was found in the liver of one specimen, an adult female (4.5 gm wet weight; 50 mm SL). The lesion was a well-circumscribed cholangioma approximately 750 x 960 µm in greatest dimension. It occupied ~2.5% of the liver area in the plane of section examined and was situated at the periphery of the liver (Figure 1). The lesion had regular, well-defined borders with no invasion into surrounding parenchyma. It consisted of numerous well-formed bile ducts possessing normal-appearing cuboidal epithelium within a minimal

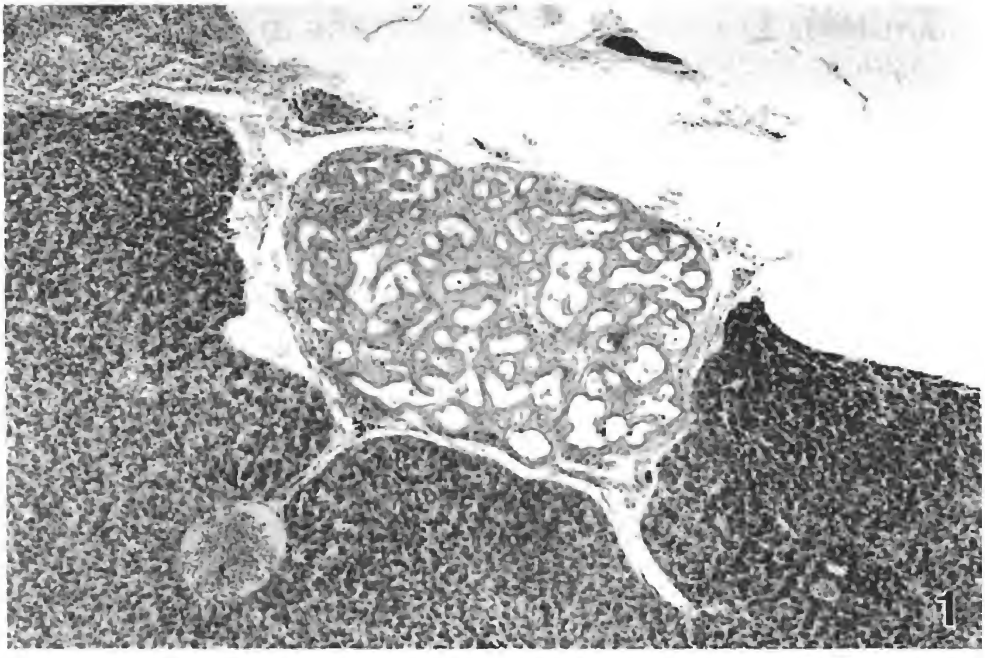

Figure 1. Low power magnification showing well-circumscribed cholangioma located at periphery of liver of a sheepshead minnow (*Cyprinodon variegatus*). (H&E; 76x).

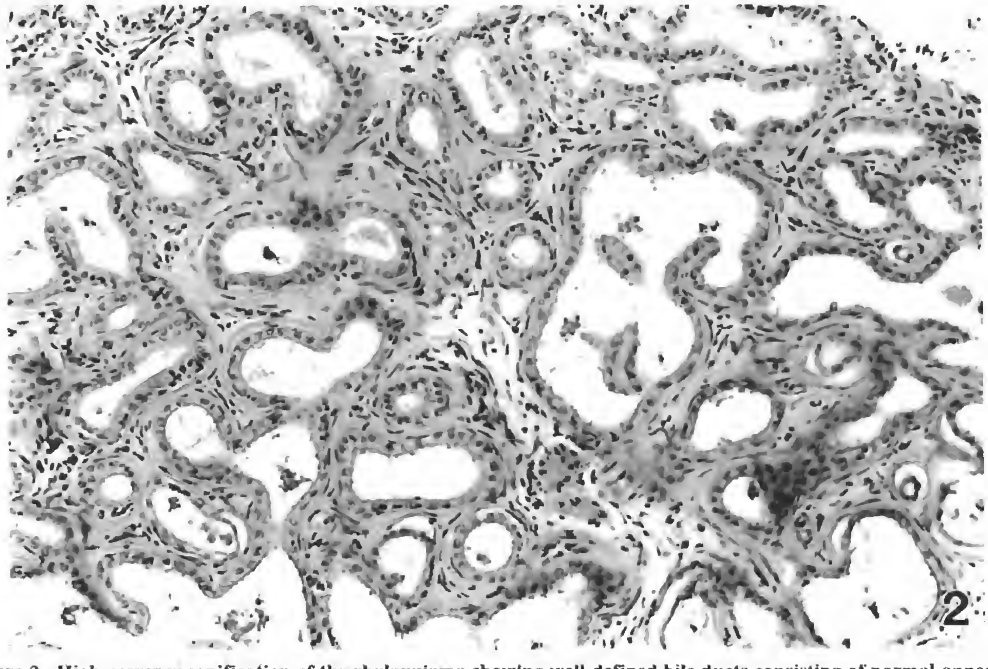

Figure 2. High power magnification of the cholangioma showing well-defined bile ducts consisting of normal-appearing cuboidal epithelium, ranging from circular to multilocular in profile and surrounded by a minimal connective tissue matrix. (H&E; 257x).

matrix of connective tissue (Figure 2). Bile duct profiles ranged from small circular configurations to relatively large multilocular structures. No mitotic figures were noted. No positive reaction with any of the P-glycoprotein antibodies tested was observed. The architecture and cellular profile of this lesion resembled that of cholangiomas described from field collections (e.g., Dawe et al. 1964; Myers et al. 1987; Bunton and Baksi 1988) and of chemically-induced cholangiomas reported from various small fish species (e.g., Hawkins et al. 1988; Grizzle and Thiyagarajah 1988).

The present case is important in that it represents only the second case of a hepatic neoplasm from a wild-caught sheepshead minnow. Furthermore, this is the first report of a biliary neoplasm from this species that was not experimentally-induced or from any other wild-caught fish species in the Gulf of Mexico. Numerous specimens from wild and laboratory-reared populations of sheepshead minnows from the northern Gulf of Mexico have been used in toxicity and carcinogenicity studies with no neoplastic lesions reported from any untreated experimental specimens (see Couch and Courtney 1987) and only one neoplasm observed from a wild specimen (Oliveira et al. 1994). These observations support a very low rate of spontaneous neoplasm development in the sheepshead minnow, an important consideration in the evaluation of results of carcinogenicity tests and in field monitoring. Furthermore, Vogelbein et al. (1990) demonstrated the significance of utilizing small fish species that have a restricted home range in monitoring for environmental carcinogens. This study showed that a few hundred meters can make a significant difference in the histology of fishes located around a point-source contamination.

Most other sheepshead minnows used at the Gulf Breeze EPA laboratory were collected at sites several kilometers west of the location where these specimens were sampled. The significance of finding one hepatic neoplasm at this site in a sample of only 10 fish histologically examined is unclear. As in the report of Oliveira et al. (1994), the present case does not constitute an epizootic, and the collection site is considered uncontaminated, with no significant commercial or residential development in its general vicinity and no other apparent sources of xenobiotic contamination. Nevertheless, this report further demonstrates the susceptibility of the sheepshead minnow to neoplastic development and supports its selection as a subject for field monitoring of carcinogenic exposure.

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First Sperm Whale (*Physeter macrocephalus*) Record in Mississippi

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FIRST SPERM WHALE *(PHYSETER MACROCEPHALUS)* RECORD IN MISSISSIPPI

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ABSTRACT A sperm whale (*Physeter macrocephalus*) stranded on the south shore of Horn Island, Mississippi, represents the first record of this species in the state. The specimen, a neonate female, was euthanized at the stranding site. Tissue samples, blood samples, and stomach contents were analyzed following gross necropsy.

Introduction

On 16 March 1994, National Park Service (NPS) rangers patrolling Horn Island, Mississippi, reported a live whale stranding on the south shore. Horn Island, part of the Gulf Islands National Seashore, lies about 10 km south of the Mississippi mainland and forms part of the boundary between the Mississippi Sound and the Gulf of Mexico (Eleuterius 1978). In the southeastern United States, the bottlenose dolphin (*Tursiops truncatus*) strands in greater frequency than all other cetacean species combined (Odell 1991). During 1993 and 1994, the National Marine Fisheries Service (NMFS) received reports of 107 stranding events on Mississippi shores. Of the 107 strandings, 103 were bottlenose dolphins. Because occurrences of cetaceans other than bottlenose dolphins in Mississippi waters are unusual (Mead 1994), the NMFS laboratory in Pascagoula, Mississippi, was contacted. Personnel familiar with marine mammal strandings mobilized at the stranding site early that afternoon. The stranded animal was a sperm whale (*Physeter macrocephalus*), and this identification is the first record of this species in Mississippi. Details of this stranding event and a summary of the biological information obtained are included in this report.

Materials and Methods

The bulbous, blunt head, asymmetrically-positioned blowhole, distinctive dorsal hump, and size identified the stranded cetacean (Field ID. MS-0008) as a small sperm whale (see Leatherwood and Reeves 1983). Despite being immobilized, the whale took regular breaths and frequently emitted low-frequency clicks and groans. Physical examination of the urogenital slit established its sex as female. The teeth were unerupted and the overall length was 610 cm from the tip of the rostrum to the notch between the flukes. These criteria placed her in the late neonate/early weaning stage of life (Geraci and Lounsbury 1993). A sperm whale at this stage would require parental care, although nothing indicated the presence of another sperm whale in the area. Based on the Von Bertalanffly equation with parameters fitted by Banister 1969 (as reported by Rice 1989) for western Australian sperm whales, it was estimated that she was about three years old. From length-to-weight formulae summarized by Lockyer 1976 (as reported by Rice 1989), her estimated weight was between 1.7 and 2.7 metric tons.

Sperm whales typically inhabit oceanic waters at depths greater than 200 meters (Jefferson et al. 1993), and are known to frequent the deep water surrounding the Mississippi River delta (Mullin et al. 1994). Horn Island lies about 135 km northeast of the Mississippi River delta and 130 km from the nearest edge of the continental shelf. For this whale to have entered the shallow waters near Horn Island, it is likely she was weak, disoriented, ill, or had followed a sick mother.

Late that afternoon, NMFS and NPS personnel managed to wrest the whale from the beach and guide her to open water. Immediately, she turned around and swam back to the beach. The process was repeated several times and it was not until a park ranger started the outboard engine of his patrol boat that she began to swim away from shore. The boat followed the whale until she was several hundred meters offshore. Visual contact with the whale was lost near dusk.

On the morning of 17 March 1994, an NPS ranger found the same whale stranded on Horn Island about 2 km east of the previous day's stranding site (30°14.11 N, 88°44.32 W). NMFS personnel joined NPS rangers and personnel from Marine Life Oceanarium, Gulfport, Mississippi, at the site. The whale's color had changed conspicuously since the previous day's examination with a reddish tinge obscuring the normal charcoal-gray color.

She was much weaker than the day before, characterized by listlessness and infrequent vocalizations. The firmly beached animal could not be moved to a rehabilitation site with the available resources. Given the high probability of the whale's eventual death, euthanasia was decided upon to prevent further suffering. A veterinarian administered 20 cc of the barbiturate *Beuthansia-D Special*®[1] in the left pectoral fin and an additional 20 cc of the barbiturate into a vein on the dorsal side of the fluke. Within five minutes of the injection, the whale had lost reflexes to her eye, a practical indication of death.

RESULTS

Standard measurements were taken at the site and are filed at the NMFS laboratory in Pascagoula. Gross necropsy performed at the site revealed multiple, crater-like lesions across the whale's body and blood in the abdominal cavity. The blood vessels in the abdominal cavity and small intestine were cyanotic, the second stomach bloated, and areas around the bile ducts yellowish. Tissue samples from major organs including skin, heart, liver, small intestine, large intestine, eye, tongue, lung, brain, kidney, and several glands were removed for pathological examination. The organs were not weighed due to the remoteness of the stranding site. Blood samples were collected. The lower jaw and additional tissue samples were collected and later placed in frozen storage at the NMFS Pascagoula laboratory. The cranium was removed and taken to the NMFS Pascagoula laboratory at a later date. Several lesions, 2-5 cm in diameter, were removed for examination. Two live fish that remained attached to the whale were placed in preservative, later identified as whale suckers (*Remora australis*), and sent to the NMFS laboratory in Miami, Florida.

Samples of skin lesions, liver, perirenal fibroadipose, and lymph node material were examined by the Armed Forces Institute of Pathology (AFIP, #2444155-2), Washington, DC. Examination detected mixed gram-negative bacilli and ciliated protozoa in the skin lesions; however, the bacilli and protozoa may have been secondary invaders and not the initiating event. AFIP examination also revealed nonspecific hepatic congestion and a fatty change in liver hepatocytes; congestion is a common, terminal, finding and the fatty change may be normal. Hemorrhaging found in the perirenal fibroadipose tissue may have resulted from trauma associated with the stranding.

Blood analysis found several abnormalities. The white blood cell count was high. Hematocrit was elevated, indicating possible dehydration. Total bilirubin and SGOT (AST), both indicators of possible liver damage, were elevated. Creatinine was elevated, indicating possible kidney damage. Lymphocytes were elevated, indicating viral infection. LDH was elevated, indicating muscle/tissue damage which may have resulted from trauma associated with the stranding. CPK, a non-specific indicator, was elevated. The erythrocyte sedimentation rate, a prognostic indicator, was elevated to 89, a poor prognosis.

The whale's stomach contents were examined at the Marine Mammal Stranding Network Southeastern United States (SEUS) laboratory in Orlando, Florida. SEUS examination found the contents to consist of cephalopod beaks and beak fragments in the forestomach and the connecting stomach. Cephalopods are the staple diet of sperm whales in most oceans (Rice 1989). This is the smallest sperm whale with cephalopod beaks in its stomach cataloged in SEUS records (Barros, personal communication). No parasites were found in the stomachs.

DISCUSSION

Sperm whales are common in the oceanic Gulf of Mexico; once the species was numerous enough to support full-scale whaling operations in Gulf waters (Townsend 1935; Gunter 1954; Schmidly 1981). Townsend 1935 (as reported by Schmidly 1981) included many records of sperm whales from April through July in the northcentral Gulf of Mexico. Recent aerial surveys of the northern Gulf of Mexico sighted sperm whales in every season of the year (Mullin et al. 1994). Stranding records compiled by Schmidly (1981) and Jefferson et al. (1992) list 22 sperm whale strandings from the Gulf of Mexico. Strandings have occurred on the shores of Florida, Louisiana, and Texas, but not on Mississippi or Alabama shores. As of 1989, the sperm whale was not listed in Mississippi records (Jones and Carter 1989).

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[1] Manufactured by Schering Plough; use does not imply endorsement.

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